Review

Overview of caffeine effects on human health and emerging delivery strategies

Sofia M. Saraiva [1], Telma A. Jacinto [1], Ana C. Gonçalves [2], Dário Gaspar [3] and Luís R. Silva [1,2,4,*]

[1] CPIRN-UDI/IPG, Center of Potential and Innovation of Natural Resources, Research Unit for Inland Development (UDI), Polytechnic Institute of Guarda, 6300-559 Guarda, Portugal. luisfarmacognosia@gmail.com (L.R.S.); sofiasaraiva@ipg.pt (S.M.S.); telmajacinto@ipg.pt (T.A.J.).

[2] CICS-UBI—Health Sciences Research Centre, University of Beira Interior, 6201-001 Covilhã, Portugal; anacarolinagoncalves@sapo.pt (A.C.G.); dariogaspar7@gmail.com (D.G.).

[3] Department of Sport Sciences, University of Beira Interior, 6201-001 Covilhã, Portugal; dariogaspar7@gmail.com (D.G.).

[4] University of Coimbra, CIEPQPF, Department of Chemical Engineering, Rua Sílvio Lima, Pólo II – Pinhal de Marrocos, 3030-790 Coimbra, Portugal.

* Correspondence: luisfarmacognosia@gmail.com; Tel.: +351-275-329-077

Abstract: Caffeine is a naturally occurring alkaloid found in various plants. It acts as a stimulant, antioxidant, anti-inflammatory and even an aid in pain management, and is found in several over-the-counter medications. This naturally derived bioactive compound is the best-known ingredient in coffee and other beverages, such as tea, soft drinks, and energy drinks, and is the most widely consumed substance worldwide. Therefore, it is extremely important to research the effects of this substance on the human body. With this in mind, caffeine and its derivatives have been extensively studied to evaluate its ability to prevent diseases and exert anti-aging and neuroprotective effects. This review is intended to provide an overview of the effects of caffeine on cancer, cardiovascular, immunological, inflammatory, and neurological diseases, among others. The heavily researched area of caffeine in sports will also be discussed. Finally, recent advances in the development of novel formulations, in the form of dietary supplements of caffeine and nanocarriers, to enhance the bioavailability of caffeine and its beneficial effects will be discussed. On the other hand, caffeine may contribute to elevated blood pressure, anemia, and migraine.

Keywords: caffeine; health benefits; athletics effects; dietary supplements; nanocarriers

1. Introduction

In current days, special attention is paid to natural molecules and their putative therapeutic effects to delay, or even prevent the occurrence of many diseases and improve the health status of the population [1]. Indeed, their ingestion is widely believed to have fewer or no adverse effects on humans than the most synthetic molecules, and they are also cheaper and easier to obtain [2-4]. Caffeine, in particular has been the subject of intense and in-depth research on the human organism regarding its health-promoting effects and possible beneficial effects on the performance of athletes, especially through its ability to improve anaerobic performance, muscular efficiency and speed, and reduce fatigue [5-9]. Caffeine is probably the most commonly ingested psychoactive substance in the world, found mainly in coffee, soft drinks, tea, cocoa and chocolate-like products, yerba matte leaves, guarana berries and some pharmaceuticals [10]. It is rapidly absorbed and distributed in all human tissues, reaching maximum plasma concentrations after 30-120 minutes after oral intake [9].

As far as we know, *in vivo* studies have already reported that caffeine stimulates the central nervous system, by acting as an antagonist of A1 and A2 adenosine receptors, promotes adrenaline release, increases dopamine, noradrenalin and glutamate levels, blood circulation and respiratory rate, mobilizes intracellular calcium stores, and alters fat and carbohydrates metabolism in the human body, by stimulating lipogenesis, thanks to its ability to inhibit phosphodiesterase enzymes [11-16].

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In addition, caffeine also increases energy, alertness, excitement and mood [17,18]. The safe dosage is up to 400 mg (5.7 mg/kg in a 70 kg person) [19]. Exceeding this dose or sudden cessation of caffeine intake may cause anxiety, insomnia, hallucinations, hypertension and headache, gastrointestinal and sleep disturbances, diuresis, tremors, palpitations and cardiac arrhythmias [20-23]. Therefore, considering its high consumption, extensive studies have been conducted to find out its full health potential. In addition, experience has been conducted with the encapsulation of caffeine, alone or in combination with other molecules, to enhance its biological activities [24-28]. Considering all these facts and knowing that health and regulatory authorities warn about the risks of caffeine in adolescents and young adults, children, lactating women and individuals suffering from cardiac and other health problems [10,12,16,22], the main objective of this review is to discuss the biological potential of caffeine for human health, highlighting its anticancer, immunological, anti-inflammatory, cardiovascular and neurological protective effects, as well as its effects on the performance of athletes.

2. Chemical structure and main natural sources of caffeine

Caffeine ($C_8H_{10}N_4O_2$; **Figure 1A**), also known as 1,3,7-trimethylxanthine, belongs to the group of methylxanthines, which are alkaloids [11,29]. Together with theobromine, its precursor, (**Figure 1B**), both are synthetised by fruits, leaves and seeds of many plants and trees to protect them from diseases and predators [22,30,31].

Regarding their structure, both compounds are carbon- and nitrogen-based molecules, composed of two purine rings, a pyrimidine ring (C5-ring), and an imidazole ring (C6-ring), both of which have two nitrogen atoms [32,33]. Their functional groups are the amide (a carbonyl group bonded to carbon and nitrogen atoms), the amine (at least, one hydrocarbon group bonded to a nitrogen atom) and the alkene (an unsaturated hydrocarbon with a double bond between two carbon atoms) [32]. Caffeine serves only as a hydrogen bond acceptor, because three of its four nitrogen atoms are methylated [34].

Although caffeine and theobromine share similarities at the physical and chemical levels, caffeine has an additional methylene group and exerts stronger central nervous system effects than theobromine [35,36].

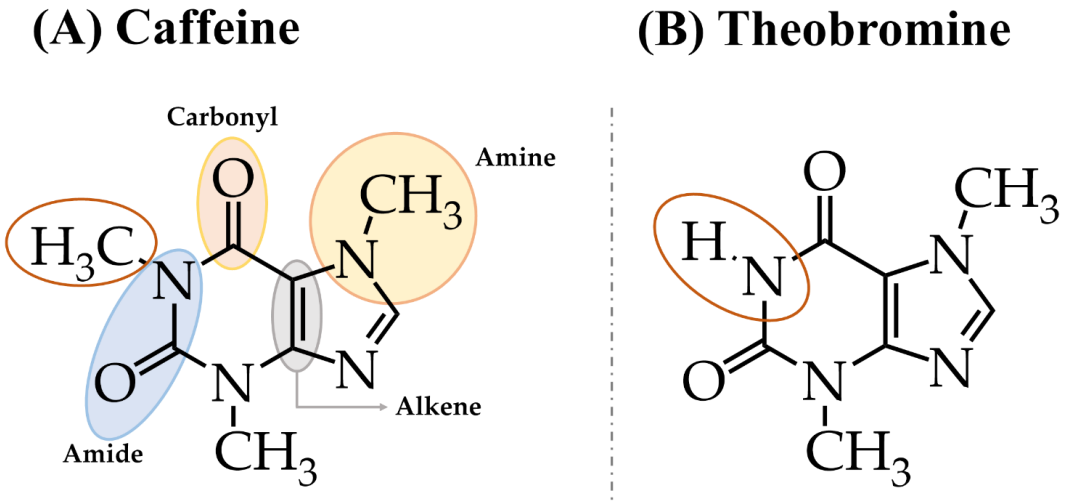

Figure 1. Chemical structure and functional groups of caffeine (A) and theobromine (B).

Regarding caffeine consumption, recent statistical data show that more than 85% of American adults consume caffeine daily (135 mg per day) [37,38]. In Europe, high caffeine consumption is observed (values ranging from 37 to 319 mg per day), especially in the Netherlands (411 mg per day), Denmark (390 mg per day), Finland (329 mg per day), Austria (300 mg per day) and Switzerland (288 mg per day) [19,39]. Brazil and Argentina also have high caffeine consumption (mean values of 40 and 100 mg per day, respectively), as well as Australia (232 mg per day) and Japan (169 mg per day)

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[39]. In contrast, low amounts of caffeine are consumed in China (16 mg per day), Angola (4 mg per day), and Kenya (50 mg per day) [39].

The main sources of caffeine are coffee and tea, energy, sodas and soft drinks, and dark chocolate (see **Table 1**) [29,39-42]. As expected, coffee and chocolate are the most important sources of caffeine worldwide [29,40]. Among the different types of coffee, American coffee is the most caffeinated (91.7-213.3 mg) [40], followed by expresso (66.0-276.0 mg) [29]. As for tea, black tea and Yerba Mate contain considerable amounts of this molecule (both around 40 mg) [39]. Among soft drinks, Mountain Dew, Guarana and Diet Coke have considerable amounts of caffeine (54.0-90.0, 47.0 and 46.0 mg, respectively) [39]. Among energy drinks, Monster and Rockstar have higher caffeine contents (amounts around 300 mg), followed by Full Throttle (160.0 mg) [39]. In addition, as expected, energy shots, such as Spike Energy Double Shot and Bang Shot are also rich in caffeine (levels of 350 and 300 mg, respectively) [39].

Finally, Cranergy juice and Water Joe also have significant amounts of caffeine (70.0 mg) [39,43]. The presence of caffeine is also reported in dark chocolate (8 mg) [37].

Table 1. Major sources of caffeine and respective levels.

Sources	Caffeine range (mg)	References
Coffee		
Americano coffee	91.7-213.3	[40]
Decaffeinated coffee (500 mL)	0.0-13.9	[41]
Instant coffee	8.7-120.0	[29,39,40,42]
Plain coffee	68.4-136.9	[40]
Espresso	66.0-276.0	[29]
Tea		
Black tea	42.0	[39]
Green tea	18.0	[39]
Yerba Mate	40.0	[39]
Soft drinks		
Coca-Cola classic	34.0	[39]
Diet coke	46.0	[39]
Guarana	47.0	[39]
Mountain Dew	54.0-90.0	[39]
Pepsi-Cola	38.0	[39]
Soda	0.0-69.0	[39]
Sunkist	19.0	[39]
Energy drinks		
Mountain Dew Amp	142.0	[39]
Full Throttle	160.0	[39]
Monster	60.0-300.0	[39]
Red Bull	80.0-106.0	[39]
Rockstar	160.0-300.0	[39]
Juice		
Cranergy	70.0-80.0	[39]
Energy shots		
Bang Shot	300.0	[39]
5 Hour Energy	200.0	[39]
TruBrain Extra	100.0	[39]
Spike Energy Double Shot	350.0	[39]
Chocolate		
Dark chocolate	8.0	[39]
Others		

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Water Joe	70.0	[39,43]

3. Benefits of caffeine on health

For our search, we used Web of Science, search restrictions were based on language (English), year of publication from 2018 to present and type of publication set to journal. The keywords used for the search were "caffeine" in combination with any of these other keywords, "cancer", "anti-cancer", "antitumor", "anti-tumor", anti-cancer", "neurodegenerative diseases", "autoimmune diseases", "immunological", "immunomodulatory", "immune system", "anti-inflammatory" and "cardiovascular". In the following subsections we provide an overview of the most recent research regarding the impact of caffeine in different illnesses such as, cancer, autoimmune diseases, immunomodulation, neuroinflammation and ocular, respiratory neurodegenerative and cardiovascular diseases.

3.1. Cancer

Cancer is one of the leading causes of death worldwide, along with cardiovascular diseases (CVD) [44]. It is estimated that over 22 million people will develop cancer in 2030 [45,46]. In addition, cancer responsible for a significant economic burden on both the health care system and patients [45].

As early as 2000, Hanahan and Weinberg defined key features (i.e. "hallmarks of cancer") that describe the characteristics necessary to promote cancer growth and metastasis [47]. In 2011, the authors revised the original hallmarks and added two morecancer-promoting features and two more hallmarks [48]. Gaascht et al. and Cadóna et al, reviewed the role of coffee components in suppressing some of the cancer hallmarks defined by Hanahan and Weinberg [49,50], while other authors fully elucidated the effect of caffeine on the cell cycle [51]. In addition, Hanahan reviewed the previously discussed features in early 2022, as the understanding of cancer underlying mechanisms of progression has grown as have the available experimental and computational tools [52]. The new additional features of cancer are (i) phenotypic plasticity, (ii) nonmutational epigenic reprogramming, (iii) polymorphic microbiomes and (iv) senescent cells [52].

For example, El Far et al., studied the effect of caffeine and other natural substances on senescent cells of colon and breast cancer. After inducing senescence with doxorubicin, cells were treated with various doses of caffeine (0, 5, 10, 15, 20, 30, 40, 50 and 60 mM). The IC_{50} of caffeine against doxorubicin treated HCT116 and MCF7 cells was 13.36 ± 2.29 mM and 17.67 ± 3.98 mM, respectively. The authors also examined caffeine-induced apoptosis in both senescent and proliferative cells. At concentrations of 10 and 15 mM, caffein induced a significant increase in apoptosis in senescent HTC116 cells, and at concentration of 5, 10 and 15 mM in senescent MCF7 cells, compared with proliferative cells [53].

In another study, Machado et. al, evaluated the effect of caffeine on two breast cancer cell lines (MCF-7 and MDA-MB-231). The results showed that caffeine at a concentration of 2.5 mM and 5 mM for MCF-7 and MDA-MB-231, respectively, reduces cell viability and induced apoptosis [54].

Venkata Charan Tej et al., investigated the effect of caffeine on the carcinogen-induced tumor model of fibrosarcoma. After 250 days of 3-MCA inoculation, there was a dose-dependent decrease in tumor incidence, size, and growth rate in the groups treated with caffeine (0.02%, 0.04% and 0.08% w/v). In addition, caffeine-treated mice had a higher percentage of cytotoxic T cells and higher TNF-α and IFN-γ levels, and finally decreased the expression of PD-1 [55].

In another *in vivo* study, Higuchi et al., evaluated the efficacy of oral recombinant methioninase (o-rMETase) in combination with caffeine and doxorubicin in an orthotopic xenograft mouse model of synovial sarcoma. After two weeks of treatment, the group treated with the combination of caffeine, doxorubicin and o-rMETase was able to induce tumor regression. According to the authors, this can be explained by the ability of caffeine to induce mitotic catastrophe [56].

Glucose-6-phosphate dehydrogenase (G6PDH) is considered a biomarker and potential therapeutic target for renal cell carcinoma. The *in silico* study conducted by Xu et al, showed that caffeine is able to bind to G6PDHConsistent with the above results, in this study, the use of caffeine, at

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concentrations of 0–3200 μg/mL for *in vitro* studies and 60 and 120 mg/kg/day for *in vivo*, studies decreased the viability and proliferation of ACHN and 786-O cell lines [57].

Therefore, it is extremely important to understand the effects of caffeine on cancer and the mechanisms underlying this interaction, which are summarized in **Table 2**.

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Table 2. Overview of the latest research regarding caffeine anti-cancer activity.

Target Cancer	Study Type	Model	Caffeine concentration	Result	Reference
Carcinoma squamous cells	*In vitro*	HN5 and KYSE30 cells	0.5 - 70 mmol	Caffeine at the concentrations of 20, 50 and 70 mmol presented an inhibitory effect and decreased the proliferation rate of both cell lines.	[58]
Glioblastoma multiforme	*In vitro*	Human GBM cell line U87-MG	1 mM	Pretreatment of cells with caffeine followed by combined treatment of Temozolomide + caffeine significantly decreased cell viability compared with the other groups.	[59]
Glioblastoma multiforme	*In vitro*	human GBM cell line U87MG and T98G 101 cells	0.5- 10	In both cell lines, caffeine at a concentration of 2.5 mM was able to reduce cellular viability, which was more pronounced under hypoxia.	[60]
Pancreatic ductal adenocarcinoma	*In vitro*	AsPC-1, BxPC-3, Capan-1, COLO-357, MiaPaCa-2, SU.86.86, PANC-1, and T3M4 pancreatic cancer cells	100, 200 μM	Caffeine enhanced cell death induced by 5-Fluorouracil and Gemcitabine, and also decreased the IC50 of both chemotherapeutic agents.	[61]
Prostate cancer	*In vitro*	PC-3 cells	0.5 mM	The caffeine affected the cell viability in a dose-dependent manner. Cell migration and invasion ability was more affected by the combination of atorvastatin and caffeine than by caffeine alone. The same was true for the formation of tumor spheres.	[62]
Melanoma	*In vitro*	Normal human melanocytes COLO829 and C32 cells	0.01 - 1.0 μmol/mL	The results show the ability of caffeine to reduce the viability of COLO829 and C32 cells, by 5-35% and 1-16%, respectively. In addition, it also led to a decrease in thiol degradation and pro-apoptotic effect and did not affect normal melanocytes cells.	[63]
Breast cancer	*In vitro*	MDA-MB-231, MCF7 and MCF10A cells	125 nM	After treatment of MDA-MB-231 and MCF7 with caffein, there was a change in metabolism towards respiratory-chain phosphorylation with low ratio of free to bound NADH. In combination with cisplatin, there was a decrease in viability, and preference of cancer cells over normal breast cells.	[64]
Colon and Breast cancer	*In vitro*	HCT116 and MCF7 cells	0 to 60 mM	Apoptosis increased in both cell lines proliferative and senescent cells after treatment of the cells with caffeine at a concentration of 15 mM.	[53]

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Lung cancer	*In vitro*	NCI-H23 and MLC15 cells	0–500 μM	After treatment of NCl-H23 cells with 250 and 500 μM caffeine, the size of colonies decreased by 78.1% and 63.9%, respectively. In addition, caffeine at the same concentrations also induced cell arrest in the G0/G1 phase, reduced the S phase of the cell cycle, and suppressed cell invasion.	[65]
Melanoma	*In vitro*	B16F10 cells	1–40 μM	Pre-treatment cells with caffeine enhanced the cytotoxic effects induced by dacarbazine. In addition, caffeine also increased oxidative-stress in a dose-dependent manner.	[66]
Breast	*In vitro*	MCF-7 and MDA-MB-231cells	1-10 mM	In MTT assay, caffeine reduced the cell viability in concentrations greater than 2.5 mM for MCF7 and for 5 and 10 mM for MDA-MB-231 cell line. At the last-mentioned concentrations caffeine induces apoptosis and necrosis in both cell lines.	[54]
Endometrial cancer	*In vitro*	RL95-2, HEC-1-A and KLE cells	0-40 mM	The therapeutic concentration of cisplatin decreased from 4.1 to 1.1 μM and from 163 to 6.6 μM, with caffeine concentrations of 1.1 and 5.3 Mm, respectively.	[67]
Glioma		RT2 cells-induced glioma in male Fischer 344 inbred rat	30 mg/kg/day	The combination of caffeine with temozolomide reduced tumor wrinkles compared between the control group and the group with temozolomide alone.	[68]
Hepatocellular carcinoma	*In vitro* and *in vivo*	SMMC-7721 and Hep3 cell lines and Male BALB/c nude mice	0-32 mM (*in vitro*) 20mg/kg/day (*in vivo*)	Caffeine decreased the viability of both cell lines and has a synergistic effect with 5-fluorouracil. In addition, tumor growth was suppressed, and tumor weight was reduced in mice treated with caffeine alone or in combination with 5-fluorouracil.	[69]
Adult pleomorphic rhabdomyosarcoma	*In vitro* and *in vivo*	RMS cells, Athymic nu/nu nude mice and cells removed to the tumor tissue	0.5 and 1 mM (*in vitro*) 100 mg/kg per day (*in vivo*)	Caffeine has been shown to enhance the antiproliferative effects of valproic acid. In the *in vivo* studies, the group treated with caffeine and valproic acid showed a reduction in tumor volume compared to the control group. This was also confirmed in the group treated with *Salmonella typhimurium* A1 receptor in combination with caffeine and valproic acid.	[70]
Osteossarcoma, fibrosarcoma	*In vitro* and *in vivo*	HOS, HT1080 and LM8 cells and athymic nude mice	0.5 mM (*in vitro*) 100 mg/kg (*in vivo)*	The combination of cisplatin and caffeine decreased ell viability compared with cisplatin alone. *In vivo*, after implantation of LM8 and HT1080 cells the combination of cisplatin + caffeine decreased tumor volume and weight.	[71]

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Melanoma	*In vivo*	Albino mice and C57BL/6J mice	0.08% w/v, daily	The authors demonstrated that in the carcinogen-induced tumor model, the groups treated with caffeine alone decreased the tumor growth rate from 5.3 mm^2/day to 2.6 mm^2/day, in combination with anti-PD1 the decrease was more pronounced (0.9 mm^2/day).	[72]
Fibrosarcoma	*In vivo*	Adult albino mice	0.02%, 0.04%, and 0.08% w/v	In caffeine-treated mice, tumor incidence, size and growth rate decreased with the increasing concentration. In addition, caffeine-treated mice had a higher percentage of cytotoxic T cells and higher TNF-α and IFN-γ levels.	[55]
Synovial sarcoma	*In vivo*	Athymic nu/nu nude mice	100 mg/kg/day	The combination of oral recombinant methioninase and caffeine reduced tumor volume.	[56]
Osteosarcoma	*In vivo*	Athymic nu/nu nude mice	100 kg/kg/day	After treatment the osteosarcoma model (patient-derived orthotopic xenograft) with cisplatinum + oral recombinant methioninase + caffeine, the decreased was most smarked compared with the other groups.	[73]
Fibrosarcoma	*In vivo*	Adult Syrian golden hamsters	100 mg/kg	Administration of metformin (500 mg/kg) and caffeine resulted in inhibition of fibrosarcoma growth.	[74]
Colorectal cancer	*In vivo and in silico*	Swiss Webster mice	50 mg/kg/day	Mice treated with caffeine alone or in combination with chlorogenic acid decreased the expression of IL-6, IL-17 and TNF-α.	[75]
Renal cell carcinoma	*In vitro* and *in vivo* and *in silico*	ACHN and 786-O cells and BALB/c nude mice	0–3200 µg/mL	The molecular docking studies demonstrated that caffeine was able to bind to G6PDH at the NADP+ binding site, which is a biomarker and potential therapeutic target for renal cell carcinoma. In addition, caffeine was able to decrease the viability and proliferation of both cell lines and in the *in vivo* studies.	[57]

IC50, half-maximal inhibitory concentration; NADH, nicotinamide adenine dinucleotide; MTT assay, (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay; Anti-PD1, anti-Programmed Cell Death Protein 1; TNF-α, tumor necrosis factor; IFN-γ , interferon gamma; G6PDH, Glucose-6-phosphate dehydrogenase; NADP+, nicotinamide adenine dinucleotide phosphate

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3.2. Anti-inflammatory and Immunomodulation

3.2.1. Autoimmune diseases and immunomodulation

Inflammation is usually caused by infection or damage to a tissue [76]. Caffeine has the ability to exert modulation on the immune system. The immune response can be divided into two types: (i) innate and (ii) adaptive immunity [77]. Acute inflammation is a mechanism of innate immunity, whereas chronic inflammation usually contributes to the development of various diseases, such as metabolic disorders, neurodegenerative diseases and even cancers [78,79]. According to several authors, the effect of caffeine on the innate immune system is due to the activity of natural killer cells, which subsequently release cytokines and eventually activate the defense mechanism. In addition, caffeine can decrease immunity the chemotaxis of neutrophils and monocytes. As for adaptive immunity, the effect of caffeine is due to the inhibition of Th1 and Th2 cell proliferation, as well as to the alteration of B cells function and the consequent suppression of antibody production [80-83]. Several authors, such as Horrigan et al. and Açıkalın et al., already reviewed, in depth, the impact of caffeine on the immune system [84,85].

Wang et al, evaluated the effects of caffeine on multiple sclerosis. Experimental autoimmune encephalomyelitis is the standard animal model for multiple sclerosis. After infecting, C57BL/6 mice with the disease, they were treated with caffeine (10, 20 or 30 mg/kg/day) in drinking water. The results showed that caffeine could reduce inflammatory cell infiltration, the degree of demyelination and microglial *in vivo*. It also reduced NLRP3 and p62 protein levels. *In vitro* assays indicated that caffein promoted autophagy. [86]. In another study, Ghaffary et al. evaluated the potential of mesenchymal stem cells to reduce the severity of rheumatoid arthritis. After the disease was induced in Wistar rats, the rats were treated with mesenchymal stem cells that had previously been incubated with various concentrations of caffeine. The results showed that the rats treated with 0.5 mM pulsed mesenchymal stem cells with caffeine decreased disease severity and serum levels of C-reactive protein, nitric oxide, myeloperoxidase and TNF-α. In addition, it increased IL-10 serum levels and weight in treated rats [87].

3.2.2. Neuroinflammation

As mentioned earlier, caffeine exerts its effects at adenosine receptors (A1 and A2A), which are generally known to play a key role in inflammatory mechanism and energy production [88,89].

Although caffeine is not selective for A2A, the effect of caffeine on this receptor has been described with different effects. On the other hand, low concentrations (up to 100 uM) of caffeine have been reported to decrease adenosine monophosphate (cAMP) production and enhance the acute inflammatory response. In addition, it has recently been shown *in vitro*, that treatment of whole blood with LPS and high concentrations of caffeine (50 uM) can increase cellular cAMP production and decrease levels of the proinflammatory TNF-α [90]. *In vivo* studies have also reported A2A activation with chronic oral administration of caffeine (0.1 to 5 g/L) to mice and reduction of pro-inflammatory cytokines production and thus inflammatory response in tissues, such as the brain [91]. In another i*n vitro* study, incubation of caffeine at concentrations ranging from 0.019 to 1.16 mM, was shown to inhibit STAT1 signalling and decrease cytokine levels of IL-8, MIP-1β, IL-6, IFN-γ, GM-CSF, TNF, IL-2, IL-4, MCP-1, and IL-10. In addition, the levels of TNF−α, an important inflammatory cytokine in immune diseases, were also reduced at protein and mRNA levels [92].

3.2.3. Ocular diseases

Adenosine receptors are expressed by retinal endothelial and retinal pigment epithelial (RPE) cells, as well as choroid and choroidal cells [93]. Therefore, caffeine may also have beneficial effects in ocular diseases, such as choroidal neovascularization and retinal inflammation. Retinal inflammation is involved in ocular diseases as age-related macular degeneration (AMD), diabetic retinopathy (DR), among others. For example, AMD is characterized by elevated vitreous levels of IL-1β [94] and

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plasmatic tumor necrosis receptor 2 (TNF-R2) and low levels of BDNF in the aqueous humor, which negatively affects photoreceptor and retinal ganglion cell survival [95].

Conti et al., demonstrated that caffeine has an anti-inflammatory effect in RPE cells, decreasing the expression of IL-1β, IL-6, and TNF-α, as well as nuclear translocation of nuclear factor kappa B (NF-κB). In addition, topical instillation of caffeine in an ischemia-reperfusion injury mice model, was shown to restore physiological BDNF levels and reduce the mRNA levels of IL-6 in the retina, demonstrating its potential for the treatment of retinal inflammation and degeneration [96]. The effect of caffeine on choroidal AR receptors, reduction of cell migration to the injured area, and angiogenesis demonstrate the importance of caffeine in attenuating choroidal neovascularization [93].

3.2.4. Respiratory diseases

Currently, there are respiratory diseases for which caffeine is used as a clinical treatment, namely premature diseases such as apnea and bronchopulmonary dysplasia (BPD). Caffeine is the most commonly used medication for extreme prematurity (less than 28 weeks) and is also very commonly prescribed for very early preterm birth (28 to 32 weeks) [97]. As clinically shown, early initiation of caffeine treatment (5 and 10 mg/kg/day) is important to achieve a successful outcome. Early treatment significantly reduced BPD incidence and mortality in low-birth-weight neonates [98]. BPD is a common neonatal pulmonary complication with a prevalence of 45% in preterm infants [99]. This multifactorial disease is associated with a nonspecific inflammatory response, involving activation of Toll-like receptors (TLRs), NOD-like receptors (NLRs), and increased levels of pro-inflammatory cytokines (IL-1β, IL-6, IL-8, IL-18, TNFα) [100]. In addition, NLR3 (NOD-, LRR- and pyrin domain-containing protein 3) a key player in the pathogenesis of BPD, is responsible for the release of pro-inflammatory cytokines (IL-1β and IL-18) and alveolar cell death through various mechanisms [101,102]. Despite the use of caffeine and its clear benefits, the mechanisms behind the clinical benefits in these diseases are not fully understood.

In vitro treatment of LPS-induced macrophages with caffeine, caused a reduction in caspase-1 expression and inhibition of the NLRP3 inflammasome, demonstrating its potential effect on this important target. Moreover, *in vivo*, treatment of newborn mice with hypoxia induced lung injury with caffeine was shown to significantly decreased A2A receptor expression and inhibit NLRP3 inflammasome protein and NF-κB pathway in the lung. The effect of caffeine on these key regulators, attenuated inflammatory infiltration, reduced oxidative stress, decreased alveolar cell death, and promoted alveolar development [103]. Similar results were also observed in another study, specifically, caffeine caused a decrease in NF-κB and pro-inflammatory factors' levels, increased the expression of A1, A2A and A2B receptors, and decreased cell death in the lung [104].

Table 3 summarizes recent research findings on the anti-inflammatory effects of caffeine and its effects on autoimmune diseases.

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Table 3. Overview of the latest research regarding caffeine anti-inflammatory activity and impact on the immune system.

Target/Goal	Study Type	Model	Caffeine concentration	Result	Reference
Anti-inflammatory effect and immunomodulation	*In vitro*	Human peripheral blood mononuclear cells	0.019 -1.16 mM	Caffeine reduced the levels of several cytokines (IL-8, MIP-1β, IL-6, IFN-γ, GM-CSF, TNF-α, IL-2, IL-4, MCP-1, and IL-10. It also inhibited STAT1 signaling.	[92]
Immunomodulation	*In vitro*	Monocytes and macrophage	300–1000 μM	Caffeine suppressed TNF−α in both LPS-activated macrophage subtypes, altered adenosine receptor expression, Akt/AMPK/mTOR signaling and inhibited STAT/IL-10 signaling in macrophage colony-stimulating factor.	[105]
Immunomodulation	*In vitro*	Mesenchymal stem cells and neutrophiles	0.1-1 mM	Caffeine-treated mesenchymal stem cells produced fewer reactive oxygen species and increased phagocytosis of neutrophils co-cultured with mesenchymal stem cells.	[106]
Immunomodulation	*In vitro*	Mesenchymal stem cells and neutrophiles	0.1-1 mM	Caffeine treatment increased the viability of co-cultured neutrophils.	[107]
Bronchopulmonary dysplasia - NLRP3 inflammasome	*In vitro*	THP-1-derived macrophages	100-800 μM	There was a decreased in NLRP3 inflammasome activation, ASC speck formation, and caspase 1 cleavage. In addition, IL-1β and IL-18 decreased secretion, and phosphorylation of MAPK and NF-kB pathway members	[108]
Melanoma	*In vitro* *in silico*	Mel1 and Mel3 cells	1 and 2 mM	After caffeine treatment, there was a decrease in the levels of IL-1β, IP-10, macrophage inflammatory protein 1-α, and CCL4. On the other hand, the expression of regulated and normal T cells decreased in Mel3 cell line.	[109]
Rheumatoid arthritis	*In vitro* and *in vivo*	Mesenchymal stem cells and Wistar rats	0-1 mM	Caffeine at a concentration of 0.5 Mm can promote lower levels of cytokines, such as IFN-γ, IL-6, and IL-1β and higher levels of IDO and TGF-β. In addition, cells treated with caffeine diminish the severity of rheumatoid arthritis and cause a decrease in serum levels of C-reactive protein, nitric oxide, myeloperoxidase, and TNF-α.	[87]
Infection	*In vitro* and *in vivo*	Peritoneal macrophages and Swiss mice	0.05- 5 μg/mL (*in vitro*) 0.05-5 mg/Kg (*in vivo*)	In mice, the leucocyte infiltration of the peritoneal cavity decreased after caffeine treatment. In addition, mRNA expressions of IL-1β, IL-6, and the enzyme inducible nitric oxide synthase were decreased, whereas IL-10 was increased.	[110]

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Immunological and metabolic anomalies in obesity	*In vitro* and *in vivo*	Male Sprague-Dawley rat, RAW 264.7 macrophage and HepG2 cells	50, 100, 150Mm (*in vitro*) 20mg/kg/day (*in vivo*)	In caffeine-treated mice, the profiles of TNF−α, MCP-1, IL-6, intercellular adhesion molecule, and nitrite were suppressed. In addition, live white adipose tissue and muscle macrophages and their cytokine levels also decreased.	[111]
Depression	*In vitro* and *in vivo*	CBA × C57BL/6 F1 mice and syngeneic splenocytes	100 μg/15 × 10^6 cells	Immune cells treated with caffeine and transplanted into depressive-like mice resulted in an increase in neuronal density and anti-inflammatory cytokines (IL-10 and IL-4) and a decrease of proinflammatory cytokine (IL-1β, INF-γ, and TNF-α).	[112]
Autoimmune Encephalomyelitis	*In vitro* and *in vivo*	Primary microglia and BV2 cells C57BL/6 mice were immunized to induce autoimmune encephalomyelitis	2mM (*in vitro*) 10-30 mg/kg/day (*in vivo*)	Caffeine decreased clinical score, inflammatory cell infiltration degree of the demyelination, and microglia stimulation in mice. In addition, it increased LC3-II/LC3-I levels and decreased NLRP3 and P62 levels.	[86]
Neurotoxicity - antioxidant and anti-inflammatory	*In vivo*	Albino rats	20 mg/kg	Reduced oxidative stress and restored TNF-α levels in cerebral tissues.	[113]
Neuroinflammation	*In vivo*	Sprague-Dawley rats	60 mg/kg/day	Caffeine/modafinil increased levels of anti-inflammatory (IL-4 and IL-10) and decreased proinflammatory (TNF-α, IL-1β) cytokines in the hippocampus. Decreased the microglial immunoreactivity and improved inflammatory response and anxious behavior.	[114]
Hepatic fibrosis - antioxidant and anti-inflammatory	*In vivo*	Hepatic fibrosis Sprague-Dawley rats	50 mg/kg	Decreased fibrosis and necro-inflammation; decreased LPAR1, TGF-β1, CTGF, α-SMA and LPAR1 expression; improved liver function	[115]
Oxygen-Induced Inflammatory Lung Injury	*In vivo*	Neonatal rats	10 mg/kg	Under hyperoxia, caffeine decreased pro-inflammatory mediators (TNF-α, IL-1α, IL-1β, IFN-γ) and NF-kB, and decreased infiltrating cells in the lung. Opposite effects were observed in normotoxic conditions.	[104]
Inflammation and adenosinergic system in cerebellum	*In vivo*	Ethanol-induced inflammation in wistar and UChB rats	3 g caffeine/L of ethanol	Caffeine modulated A1 and A2a receptors and attenuated the inflammation, demonstrating a neuroprotective role.	[116]

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Choroidal neovascularization - anti-inflammatory and na	*In vitro in vivo*	Laser photocoagulation mice model	200, 400 uM (*in vitro*); 10 and 20 mg/kg (*in vivo*)	Significantly reduced the migration of retinal and choroidal endothelial cells (*in vitro*); Decreased choroidal neovascularization and inflammatory (mononuclear phagocytes) cells' recruitment to the lesion area.	[93]
Neurotoxicity	*In vivo*	Tramadol-induced damage in cerebelum rat model	37.5 mg/kg	Up-regulated autophagy-related genes; reduced the expression of inflammatory and apoptosis markers, demonstrating neuroprotective effects in the cerebellum.	[117]
Retinal inflammation	*In vitro in vivo*	Ischemia reperfusion (I/R) injury mice model	1 - 100 uM (*in vitro*); 10uL at 1,9% *(in vivo*)	Caffeine reduced the secretion of IL-1β, IL-6, and TNF-α and restored the integrity of retinal cell monolayer (*in vitro*). Instilled caffeine reduced IL-6 mRNA levels and maintained BDNF physiological levels in the retina	[96]
Cognitive impairment	*In vivo*	BALB/c mice	0.05 and 0.1 mg	Intranasal administration of caffeine improved the behavior outcomes of ischemic mice and reduced the expression of proinflammatory biomarkers (TNF-α, IL-6) and improved anti-inflammatory cytokines' (IL-10).	[118]
Hydrocephalus	*In vivo*	Kaolin-induced hydrocephalus mice	50 mg/kg by gavage (dams)	Administration of caffeine to dams reduced cell death, and increased the neurons dendritic arborization in the sensorimotor cortex and striatum of the mice neonates and improved hydrocephalic deficits and behavioral development	[119]
Anti-inflammatory effect	*In vivo*	Albino rats	100mg/kg	Administration of 100 mg/kg/day alone or in combination with nicotine decreased the number of CD68+ve macrophages and the density of CD68 immunoexpression. In addition, combined administration of caffeine and nicotine decreased apoptosis compared with nicotine alone.	[120]
Immunomodulation and anti-inflammatory effect	*In vivo*	Nile tilapia	Diet containing 5 and 8%	Diets containing 5% and 8% caffeine prevented alterations caused by hypoxia, such as ATP hydrolysis and consequent accumulation in the extracellular environment.	[121]
Dental pain	Clinical Trial	Patients with acute postoperative dental pain	100 mg	Caffeine improved the effect of ibuprofen in the treatment of moderate postoperative dental pain.	[122]

IL, interleukine; TNF-α , tumor necrosis factor; IFN-γ , interferon gamma; MCP-1 (Monocyte chemoattractant protein-1); STAT1, Signal Transducer and Activator of Transcription 1; Akt, Protein kinase B; AMPK, adenosine monophosphate-activated protein kinase; mTOR, mammalian target of rapamycin; NLRP3, NLR family pyrin domain containing 3; NF-kB, Nuclear factor-κB MAPK, mitogen-activated protein kinase; IP-10, Interferon gamma-induced protein 10; CCL4, CC motif chemokine ligand 4; TGF-β, transforming growth factor beta; CTGF, connective tissue growth factor; α-SMA, alpha smooth muscle actin; LPAR1, lysophosphatidic acid receptor 1; LPS, lipopolysaccharide; M-MFs,

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inflammation resolving macrophages; GM-MFs inflammation; promoting macrophages; NFκB1, nuclear factor kappa B subunit 1; HMGB1, High mobility group box 1 protein; BDNF, brain-derived growth factor.

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3.3. Neurodegenerative Diseases

By 2050, the number of dementia cases is estimated to be 36.5 million [123]. There are several neurodegenerative diseases, such as Parkinson's disease (PD), Alzheimer's disease, Huntington's disease and multiple sclerosis [124,125]. For example, PD is triggered by the loss of neurons, which leads to a decrease in dopamine levels. In Alzheimer's disease, there is deposition of extracellular deposits of amyloid-beta peptides and neurofibrillary tangles [126,127]. Caffeine is considered the most widely consumed psychoactive stimulant in the world. This natural compound is able to cross the blood-brain barrier [128,129] and, according to the literature, may exert a stimulant effect on the central nervous system by modulating several molecular targets, such as (i) antagonism of adenosine receptors, (ii) promotion of intercellular calcium mobilization, (iii) inhibition of phosphodiesterase and (iv) inhibition of GABAA receptors [130-133]. However, with the exception of blockade of adenosine receptors and consequent inhibition of neurotransmitter-induced signalling pathways, the other mechanisms exert their effects only at toxic concentrations of caffeine [128,130-132]. In 2022, Ruggiero et al., revised some studies on the protective effects of caffeine in various neurodegenerative diseases [134]. Some studies emphasized the neuroprotective role of caffeine. For example, Manolo et al., showed that caffeine, at a concentration of 10 mM, is able to protect 96% of the dopaminergic neurons. Co-administration of olanzapine and caffeine did not result in neuroprotection, implying that both dopamine D2-like, and Adenosine receptors (A2Ars) are required for neuroprotection [135]. In an *in silico* study of Parkinson's disease, the authors demonstrated that caffeine has the ability to bind to both wild-type and mutant parkin protein [136]. Mutation of parkin protein is the most common cause of Parkinson's disease, as is abnormal secretion and accumulation of α-synuclein [137,138]. This last part was detected in the following *in vivo* studies. Luan et al., investigated whether caffeine could protect against mutant α-synuclein-induced toxicity. Treatment exposing mice to 1g/L of caffeine in drinking water, attenuated apoptotic neuronal cell death, microglia and astroglia reactivation, culminating in syncucleinophaty [139]. In a similar study, Yan et al., investigated synergetic neuroprotection between caffeine and eicosanoyl-5-hydroxutryptamide. Both compounds, which are present in coffee, showed no effect at subtherapeutic doses, whereas their combination reduced the accumulation of phosphorylated α-synuclein, and maintained neuronal integrity and function [140].

Table 4 summarizes recent research on the neuroprotective effects of caffeine in neurodegenerative diseases and other conditions.

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Table 4. Overview of the latest research regarding caffeine on neurodegenerative diseases.

Disease	Study Type	Model	Caffeine concentration	Result	Reference
Parkinson's Disease	*In vitro*	Transgenic *Caenorhabditis elegans*	10 mM	Caffeine was able to prevent neuronal cell loss in 96% of dopaminergic neurons.	[135]
Alzheimer's Disease	*In vitro*	SHSY5Y cells	0.6 and 1 mM	Both concentrations were able to reduce beta-amyloid neurotoxicity.	[141]
Alzheimer's Disease	*In vitro*	SH-SY5Y wildtype and N2a cells	100 μM	In the presence of caffeine, the level of ADAM10 protein increased to 138.5% ± 9.2%, and the levels of APP protein level and ROS decrease to 85.4% ± 3.6% and 48.8% ± 3.2%, respectively.	[142]
Alzheimer's Disease	*In vitro*	HEK293 cells	0.1-10 mM	Caffeine induces conformational chances on muscle nicotinic acetylcholine receptors, which are molecular targets of Alzheimer's disease.	[143]
Parkinson's Disease	*In vitro* and *in vivo*	Swiss mice and Wistar rats	31.2 mg/kg	Caffeine administration reduced the catalepsy index and increased the number of ipsilateral rotations.	[144]
Cd-induced neurodegeneration	*In vitro* and *in vivo*	HT-22 and BV-2 cells and Wild-type C57BL/6N male mice	30 mg/kg/day	Caffeine reduced ROS, lipid peroxidation and 8-dihydro-8-oxoguanine levels. It also attenuated neuronal loss, synaptic dysfunction, and learning and cognitive deficits.	[145]
Hypoxia Ischemia	*In vivo*	Spague-Dawley mice	0.3 g/L	Pretreatments with caffeine reduced the brain infarct after hypoxia ischemia and also restored the brain activity.	[146]
Acetaminophen induced neurotoxicity	*In vivo*	Swiss albino mice	20 mg/kg	Treatment with Caffeine and acetaminophen reduced the formation of ROS, compared with the acetaminophen group. In addition, the survival time of caffeine- treated mice increased 33%.	[147]
Parkinson's Disease	*In vivo*	C57BL/6 mice with motor behavioral deficit induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine	20 mg/kg	Caffeine improved behavioral and neurotransmitter recovery against the induced toxicity. It was also able to restore antioxidant levels and suppress neuroinflammation.	[148]
Hypoxic-ischemic	*In vivo*	Wild type C57/bl6 specific pathogen-free mice	5 mg/kg	Caffeine administration after hypoxia ischemic brain injury reduces the lesion in the grey and white matter, and the number of amoeboid microglia and apoptotic cells. The expression of pro-inflammatory cytokines also decreased.	[149]

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Apnea of prematurity	*In vivo*	infection-free pregnant Sprague Dawley rats	100 mg/kg	Caffeine administration in normoxia, reduced the oxidative stress, hypermyelination and increase golgi bodies.	[150]
Parkinson's Disease	*In vivo*	C57BL/6 male mice	1 g/L	Caffeine protected against synucleinpathy by modulating α-syn-induced apoptosis, microglial and astrocytic activation in the striatum.	[139]
N/D	*In vivo*	male Swiss mice	0.3 g/L	The amount of A2A receptors was decreased in hippocampus of mice that consumed caffeine. The aged mice treated with caffeine presented more pyknotic neurons in the hippocampus and reduced damage.	[151]
LPS-Induced Oxidative Stress and Neuroinflammation	*In vivo*	C57BL/6N male mice	3 mg/kg/day	The LPS-injected group had enhanced expression of Bax and caspase-3. On the other hand, these markers were reduced in the group treated with caffeine, this treatment also caused a restoration of the synaptic markers.	[152]
Diabetes	*In vivo*	Male GK and Wistar-Hannover-Galas rats	1 g/L	Caffeine prevented the GFAP, vimentin and SNAP25 alterations caused by diabetes, and also improved the memory deficits.	[153]
N/D	*In vivo*	C57bl\6j mice and A2AR knockout mice	50 μM	Caffeine increased synaptic transmission by 40%, decreased facilitation of paired-pulse and decreased the amplitude of long-term potentiation by 35%.	[15]
Alzheimer Disease	*In vivo*	Wild-type N2 and CL2006 worms	200 and 400 μM	Treatment prevented amyloid beta-peptide paralysis, decreased acetylcholinesterase activity, and decreased amyloid beta-peptides mRNA levels.	[154]
Parkinson's disease	*In vivo*	C57BL/6J mice	50 mg/kg/day	Co-administration of caffeine and eicosanoyl-5-hydroxytryptamide resulted in decreased accumulation of phosphorylated α-synuclein, maintenance of neuronal integrity and function, reduction of neuroinflammation, and improvement of behavioral performance.	[140]
Parkinson's Disease	*In silico*	Molecular Docking Simulations	N/A	Caffeine was able to bind at position 28th in both wild-type and mutant parkin protein.	[136]
Alzheimer's Disease	*In silico*	Molecular Docking Simulations	N/A	The results revealed that in the presence of caffeine, the distances between the inter-residual increased, leading to the breakdown of hydrophobic contacts, and ultimately destabilizing the Aβ protofibrils.	[155]

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Parkinson's Disease	Clinical trial	Parkinson's Disease Patients	100 mg	Caffeine treatment reduced the number of errors in patient and controls on the Stroop and Choice reaction time and enhanced dual item accuracy on the rapid visual serial presentation task.	[156]

ADAM10, A Disintegrin and metalloproteinase domain-containing protein 10,; APP, Amyloid-beta precursor protein; ROS, reactive oxygen species; LPS, Lipopolysaccharides; GFAP, Glial fibrillary acidic protein; SNAP25, Synaptosomal-Associated Protein, 25kDa; N/D, non-disclosed; N/A, non-applicable.

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3.4. Cardiovascular diseases

Cardiovascular diseases, the leading cause of mortality, accounted for 17.8 million deaths worldwide between 1980 and 2017 [157]. By 2030, an estimated 23.6 million people per year will die due to CVD. Caffeine intake, particularly through the consumption of coffee, tea and other products, has shown various cardiovascular effects. Turnbull et al., reviewed more than 300 studies regarding the effects of caffeine on cardiovascular health, published from late 80s to 2017. Overall, the results suggest that caffeine consumption does not increase the risk of CVD and may have a protective effect against this group of diseases [158]. However, recent studies on this topic have shown that high caffeine consumption may have an opposite effect.

A study of 347,077 people (UK Biobank) concluded that coffee consumption may modestly increase the risk of cardiovascular disease. A nonlinear association was found between long-term coffee consumption and cardiovascular disease. Individuals who consumed coffee in high doses (> 6 cups/day) were more likely to develop cardiovascular disease (22%) than those who consumed less coffee (1-2 cups/day) [159]. In addition, the authors examined the association between coffee consumption, plasma lipids and CVD risk in 362,571 individuals (UK Biobank). The results showed that high coffee consumption (> 6 cups/day) may increase CVD risk by increasing the levels of low-density-lipoproteins cholesterol (LDL-C), total cholesterol (total-C) and apolipoprotein B (ApoB) [160].

However, other studies have reported the potential beneficial effects of moderate coffee consumption, in line with Turnbull literature review. A study of 20,487 subjects concluded that moderate coffee consumption (3-4 cups/day) was associated with a low risk of CVD-related mortality. In addition, the authors found an inverse correlation between NTproBNP levels (N-terminal fragment of the B-type natriuretic peptide, which is associated with higher stroke risk) and coffee consumption [161]. Similarly, a study of more than 500,000 participants in England, reported that intake of 121-182 mg/day of caffeine from coffee (2-3 cups/day) or tea (4-6 cups/day) consumption was associated with a low risk of coronary artery disease [162]. In addition, a U.S. follow-up study of 23,878 participants over 16 years, found that higher caffeine consumption (100-200 mg or >200 mg/day) was associated with a lower risk of CVD mortality [163]. An inverse association between coffee consumption and CVD risk factors (blood pressure and arterial stiffness) was also observed in another study, showing the beneficial effect of moderate coffee consumption [164]. A similar association was observed in relation to coffee consumption and hypertension risk [165].

Despite some studies linking high coffee or caffeine consumption to CVD risk, most studies have reported that moderate consumption has potentially beneficial and even protective effects on CVD.

Table 5 summarizes recent research on the effects of caffeine on cardiovascular diseases.

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Table 5. Overview of the latest research regarding caffeine' impact on cardiovascular diseases.

Study Type	Model	Result	Reference
Systematic review	Review of prospective studies	Regular and moderate coffee consumption (1-2 cups/day) is not associated with hypertension risk. Higher coffee consumption has a protective effect.	[165]
Prospective	347,077 volunteers (37–73 years old, UK Biobank)	Coffee consumption may lead to a slight increase in CVD risk.	[159]
Prospective	2278 volunteers (18-80 years old)	Caffeine metabolites are responsible for lowering the risk of hypertension.	[166]
Prospective	20,487 (35-94 years old)	Coffee moderate consumption (3–4 cups/day) has been associated with a lower CVD mortality.	[161]
Prospective	>500 000 individuals (40-69 years old)	The consumption of 2-3 cups of coffee per day (121-182 mg caffeine/day) was associated with a low risk of coronary artery disease.	[162]
Prospective	23,878 individuals (> 20 years old)	Higher caffeine intake (>100 mg/day) was associated with lower CVD mortality.	[163]
Prospective	362,571 individuals (37-73 years old, UK Biobank)	High coffee consumption (> 6 cups/day) increases levels of low-density-lipoproteins cholesterol, total cholesterol and apolipoprotein B, thereby increasing the risk for CVD.	[160]
Prospective	1095 individuals (mean age 53±14 years old)	Moderate coffee consumption (> 3 cups/day) reduces CVD risk factors such as arterial stiffness and high blood pressure	[164]
Randomized Controlled Trial	12 volunteers (19-39 years old)	Administration of caffeine (200 mg, 12h intervals) during sleep deprivation reduced HR and increased HF-HRV. The concentration-effect was non-linear. No significant interaction between sleep deprivation and caffeine intake	[167]
In vitro *in vivo*	Primary human and mouse aortic VSMCs, immortalized mouse aortic VSMCs; restenosis mice model (apoe−/−C57BL/6 J)	Caffeine induced autophagy by inhibiting mTOR signaling; decreased proliferation of VMCs by inhibiting WNT signaling; decreased vascular restenosis	[168]
In vivo	Zebrafish	Both concentrations tested caused a similar decrease of the HR.	[169]

HR, heart rate; HF-HRV, heart rate variability; mTOR, mammalian target of rapamycin; VSMCs, vascular smooth muscle cells.

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4. Caffeine impact on sports performance

Coffee's best-known constituent, caffeine, is the most widely consumed psychotropic drug in the world, with an estimated daily intake of up to 4mg/kg body weight in American adults [170-173]. It is a psychostimulant that can lead to physical dependence [174]. Caffeine intake is widespread among inactive individuals and high-performance athletes, especially since 2004, when it was removed from the World Anti-Doping Agency's list of banned substances for competition [175]. It is also readily available in various forms such as capsules, powders, caffeinated beverages, and energy drinks [170].

However, it is not clear in science that caffeine improves athletic performance [170-177]. Some studies show ergogenic effects on aerobic endurance (>90min), high-intensity efforts (20-60min), muscular endurance, sprint performance and maximal strength (0 to 5min), ultra-endurance (>240min) and endurance races with prolonged intermittent sprints (group sports), while others report give no evidence for its administration [176,177]. We assume that an ergogenic substance is a substance used with the aim of improving athletic performance and promoting recovery after exercise by delaying fatigue.

The word is of Greek origin, ergo (work) and gen (generation). As a result, it is commonly consumed by athletes, research suggests that 75 to 90% of athletes consume caffeine before or during athletic competition [177]. In an analysis of 20,686 urine samples from elite athletes, 73.8% of the samples contained caffeine at concentrations greater than 0.1μg/mL, suggesting that three out of four athletes consume caffeine before or during competition [172].

It should be recalled that the consumption of is not prohibited for athletes, with the maximum allowable concentration being 12mg/L of urine (International Olympic Committee). The fact that caffeine affects the nervous system, adipose tissue, and skeletal muscle originally led to the hypothesis that caffeine might affect athletic performance. For example, caffeine may increase skeletal muscle contractile force at submaximal contraction and increase the athlete's pain threshold or perceived exertion, which could lead to longer training sessions [176,177].

In addition to improving performance, there are several meta-analyses that point to the beneficial effects of caffeine on a number of chronic diseases, including cardiovascular and metabolic disorders, cancers, neurological diseases and all-cause mortality [174,177]. Interestingly, a study is presented stating that it is a common misconception that moderate coffee and tea consumption is safe in patients with cardiovascular disease. In fact, numerous studies have reported a beneficial role of moderate coffee or tea consumption in the prevention and treatment of cardiovascular disease [174].

However, it should be remembered that the caffeine intake has several side effects. Blood pressure increases both at rest and during exercise, heart rate increases, stress levels may increase due to impairments or changes in fine motor skills and technique, and it may impair recovery and sleep patterns, most likely in athletes who do not regularly consume caffeine [176]. For example, in a recent study using a caffeine dose of 12 mg/kg, almost all participants reported side effects such as tachycardia and palpitations, anxiety or nervousness, headache, and insomnia [172].

However, according to our research, it seems important to us to better evaluate certain aspects to achieve better scientific clarification with implications for practice, such as: Ideal dosage; time of intake; abstinence; training time Vs caffeine consumption; physiological factors; gender; caffeine users or not.

4.1. Optimal dosage

Higher than ideal caffeine doses, 3-6 mg/kg, before exercise do not further improve athletic performance. Additional and higher doses of caffeine may can lead to side effects in athletes [176].

Low doses of caffeine (~200 mg) have also been shown to improve attention, alertness, and mood, and cognitive processes during and after strenuous exercise. Thus, the ergogenic effects of low doses of caffeine appear to be due to changes in the central nervous system [176].

The generally accepted dosage of caffeine for performance enhancement is between 3 and 6mg/kg, 60 minutes before exercise [172].

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Although included meta-analysis report that caffeine intake can be ergogenic in a variety of physical activities, the "optimal" caffeine dose remains difficult to determine [175].

4.2. Timing of intake

Early ingestion of caffeine prior to physical activity has been shown to enhance performance. For example, caffeine can improve performance during high-intensity sprints when taken 30 minutes before exercise [173].

Because caffeine has so many positive effects on exercise performance, it can – and perhaps should – be taken before or during exercises. For most sports, it is recommended that caffeine be taken about 60 minutes before the start of the first set of the training session if used before exercise. This time-period varies depending on the individual, the type of event, and type of caffeine ingested, with caffeinated mouthwashes and chewing gums generally requiring much less time. For longer training sessions, there is evidence that ingesting caffeine later in the day, and at lower doses, may be effective [177].

Isolated consumption of anhydrous caffeine results in maximal plasma peaks of the substance between 30 and 90 minutes after consumption of low doses (2–3mg.kg−1), moderate (3–6mg/kg), or high doses (6–9mg/kg) [172,178].

4.3. Abstinence

It appears that short-term, caffeine withdrawal before competitions does not enhance the ergogenic effects of caffeine in habitual users. Withdrawal is associated with numerous negative consequences, including headache, fatigue, irritability, muscle pain, sleep disturbance, and nausea. However, these acute withdrawal symptoms, shortly before important competitions, may have a negative impact on the subjective self-confidence and well-being of the athlete [177].

4.4. Training time Vs Caffeine consumption

Increases in physical performance as a function of training time have been demonstrated in various sports. Studies suggest that anaerobic and aerobic activities may be more powerful due to the diurnal fluctuations of the circadian cycle between 4 and 8 pm. Morning caffeine consumption had a more beneficial effect than afternoon consumption [172].

4.5. Physiological factors

Hypothetically, the potential performance enhancement from caffeine ingestion may be greater in trained individuals than in untrained individuals because trained individuals have an enhanced neuromuscular action potential. Trained individuals have a higher concentration of adenosine A2a receptors than untrained individuals [172,179].

The main finding of this review is that very low doses of caffeine (>1-2 mg/kg, generally taken 60 min before exercise) improve resistance training performance, in terms of muscle strength, muscle endurance and average speed [171].

Aerobic endurance appears to be the sport in which caffeine consumption most consistently produces moderate to large benefits, although the magnitude of the effect varies among individuals [179].

4.6. Gender

Caffeine ingestion positively affects resistance exercise performance in women, and the magnitude of these effects appears to be comparable to those observed in men [178].

4.7. Caffeine consumers or not

For the first study using a performance test, 17 moderately trained men were recruited, eight of whom did not routinely consume caffeine (< 25 mg/day) and nine of whom regularly consumed

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caffeine (> 300 mg/day). It was found that there were no differences between the groups in time to exhaustion at any of the doses, suggesting that habitual caffeine consumption does not attenuate the ergogenic effects of caffeine [177]. In another study, on cycling, habitual caffeine intake was found to have no effect on athletic performance, suggesting that habituation to caffeine has no negative effect on caffeine ergogenesis [180].

5. Nanotechnology-based delivery strategies

Caffeine is usually consumed through ingestion of beverages, especially coffee, tea, and pharmaceuticals, which allows for rapid absorption and distribution in all tissues [9]. However, caffeine has a short half-life (3-5h) [181]. In addition, the oral intake of high concentrations of caffeine may cause gastrointestinal problems [182] and its wide distribution may cause undesirable side-effects, such as stimulation of the nervous system. Nanotechnology is now frequently explored for drug delivery to a target tissue. Nanocarriers offer other important advantages for caffeine delivery, namely high loading capacity, co-encapsulation of different drugs, controlled and sustained release, high surface area allowing greater interaction with tissue, and high ability to permeate through tissue [183]. In addition, other routes of administration besides oral administration can be used, such as intranasal [184] and dermal [181] (**Figure 2**).

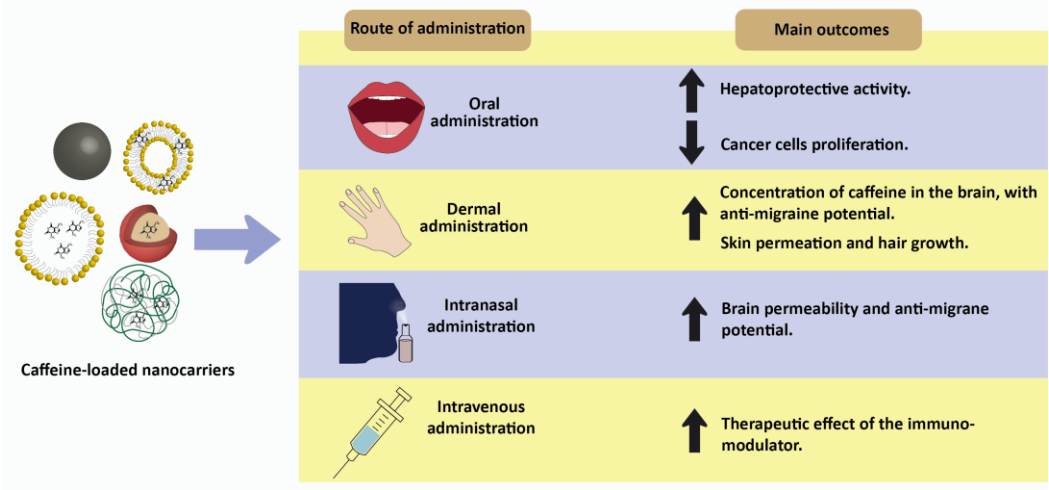

Figure 2. Possible administration routes for caffein-loaded nanosystems and their main outcomes.

Nanocarrier compositions are tailored depending on the drug(s), route of administration, and target tissue. Therefore, different nanocarrier compositions based on lipids, polymers, or metals have been proposed for caffeine delivery.

Lipidic nanocarriers have been widely explored for topical drug delivery, through the skin for cosmetic and pharmaceutical applications [185]. The composition of lipid carriers is an important factor to be considered to improve skin permeation and therapeutic effects. For example, among the various phospholipids ((1,2-distearoyl-snglycero-3-phosphocholine (DSPC), 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol, sodium salt (DPPG), 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC), and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC)) tested for liposome preparation and topical delivery of caffeine, DPPG was the most promising. Ex vivo studies showed that DPPG was able to enhance the permeation of encapsulated and free caffeine through hairless rat skin, by disrupting the lipid barrier of the stratum corneum (SC) [186]. A similar effect was observed for lipid nanocapsules (NCs) in porcine skin. The ability of lipid NCs to increase skin permeation of free caffeine, has been attributed to the combination of several factors, namely the occlusion effect of nanoparticles on the skin surface, accumulation in hair follicles and the effect on barrier function of SC [187]. On the other hand, the incorporation of propylene glycol into phosphatidyl liposomes has been shown to enhance the permeation of caffeine through the skin, as demonstrated *ex vivo* in human full-thickness skin [188]. In this sense, the researchers proposed the combination of the lipolytic activity of caffeine with

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the increased permeation capacity of propylene glycol liposomes, as a noninvasive treatment for cellulitis [188]. Amasya et al., also proposed semisolid lipid nanoparticles as a promising treatment for cellulitis because they can penetrate the skin and reach adipose tissue [189].

On the other hand, flexible liposomes composed of phosphatidylcholine and higher surfactant content (polysorbate 80 and polysorbate 20) for the treatment of alopecia by topical application [190]. The therapeutic potential of caffeine in alopecia is due to its ability to inhibit 5-α-reductase and phosphodiesterase and increase vasodilatation and blood supply to hair follicles [191]. The nanocarriers co-encapsulating minoxidil and caffeine resulted in an increase in hair length, comparable to the aqueous solution of the drugs and the commercial alcoholic solution. Nevertheless, liposomes loaded with caffeine and minoxidil led to a significant increase in hair weight, an indicator of healthy and strong hair, demonstrating the potential of liposomes for the treatment of hair loss [190]. Other types of nanosystems, namely nanoemulsions containing eucalyptol and oleic acid, have been shown to accumulate in hair follicles, and increase caffeine retention in these structures, demonstrating the potential of these nanosystems for the treatment of alopecia. Considering that hair follicles are nourished by blood vessels, targeted accumulation in these structures may enhance the permeation of caffeine. Therefore, it can also be used to develop novel therapeutics for diseases of other tissues, to avoid systemic or oral delivery.

In this sense, proniosomes have been proposed for the treatment of migraine by topical application of caffeine. As expected, caffeine-loaded proniosomes, applied topically to Swiss albino mice, were able to penetrate the skin. Moreover, topically administered caffeine-proniosomes were able to achieve significantly higher caffeine concentration in blood and brain, as well as prolonged and sustained effects, compared with orally administered caffeine solution [181]. Recently, co-delivery of caffeine and ergotamine to the brain by intranasal administration (olfactory route) has also been proposed. Hybrid lipid-polymer nanoparticles of lecithin, poly(lactic-co-glycolic acid) (PLGA), and 1,2-dipalmitoyl-sn-glycero-3-phosphocholine functionalized with polyethylene glycol (PEGylated DPPC) showed a high encapsulation efficiency (87%) and controlled release over a period of 48-hours. In addition, the results showed that the nanoparticles have high targeting accuracy in the brain without causing toxic effects. Furthermore, the synergistic effects of the drugs enhance the anti-migraine effect [184].

The anti-cancer effect of caffeine was also enhanced by the use of nanocarriers. Liu et al. prepared lipid-based nanosystems for the co-delivery of caffeine and imiquimod. Caffeine enhanced the therapeutic effect of the immunomodulator imiquimod and radiotherapy, in an orthotopic breast cancer model. The authors suggested that this may be due to modulation of the tumor microenvironment [192]. On the other hand, polymer-based nanocarriers, i.e., gelatin nanoparticles loaded with caffeine, showed the ability to decrease the viability and proliferative capacity of murine melanoma cells' (B16F10) without causing significant cytotoxic effects in normal fibroblast cells (L929) [193]. Other studies have reported the combination of caffeine with metallic nanocarriers, for example, silver-caffeine complexes anchored to magnetic nanoparticles have been proposed for the treatment of hepatocellular carcinoma [194]. This type of cancer is known to be resistant to radiotherapy and chemotherapy and can be caused by hepatitis-related infections. The most promising nanoparticles showed higher cytotoxicity against the cancer cells (hepatocellular carcinoma cells, HCC) than against the normal cells (normal hepatic cells, WRL-68). On the other hand, the targeted hyperthermia effect of the magnetic nanoparticles can improve the anti-tumor effect of the formulation and avoid the side effects of the commonly used therapeutics. In addition, these silver-caffeine-magnetic nanoparticles also showed antibacterial activity against Escherichia coli, Staphylococcus aureus and Bacillus cereus.

In addition, these silver-caffeine-magnetic nanoparticles also showed antibacterial activity against Escherichia coli, Staphylococcus aureus and Bacillus cereus [194]. Other caffeine-metal nanoparticles have been developed for antibacterial applications. Khan et al., [195] demonstrated the ability of caffeine-gold nanoparticles to inhibit biofilm formation and eliminate mature biofilms. In addition, the nanoparticles showed antibacterial activity against resistant pathogenic bacteria Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Listeria monocytogenes), demonstrating their potential for treating chronic infections.

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Overall, the different types of nanocarriers have shown the potential to improve the therapeutic effect of caffeine.

Table 6 provides an overview of recent research on the development of lipid-, polymer- and metal-based nanocarriers loading caffeine for biomedical applications.

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Table 6. Overview of the latest research regarding nanocarriers loading caffeine for biomedical applications.

Nanosystem	Method	Composition	Application	Model	Result	Reference
Lipid-based nanosystems						
Liposome	Thin-film hydration	Lecithin, polysorbate 80, polysorbate 20	Alopecia	Wistar rats	Improves skin delivery, weight, and hair length.	[190]
Liposomes	Thin film hydration	Phospholipid, cholesterol	Skin drug delivery	abdominal skin of WBN/ILA-Ht hairless rats	DPPG liposomes enhanced skin penetration by disrupting the lipidic barrier of stratum corneum.	[186]
Liposomes	High-pressure homogenization	Phosphatidylcholine, propylene glycol	Skin drug delivery	full-thickness abdominal human skin	Propylene glycol increased liposome deformability and improved skin permeation of caffeine.	[188]
Lipidic nanosystem	High-pressure homogenization	Trilaurin, oleic acid, pluronic F68, imiquimod	Cancer	orthotopic breast cancer mice model	Caffeine slightly improved antitumor activity.	[192]
Lipid nanocapsules	Phase inversion temperature	Miglyol 812 N, Kolliphor HS 15, Phospholipon 90G	Skin drug delivery	porcine skin	Caffeine was not successfully encapsulated. Nanocapsules improved the transdermal permeation of caffeine.	[187]
Semi-solid nanostructured lipid carriers	Two-stage homogenization method, high shear homogenization, ultrasonication	Compritol® 888 ATO and Precirol® ATO 5, argan oil, Poloxamer 407	Cosmetics, skin drug delivery	Wistar rat full-thickness dorsal skin	NLCs' exhibited a high capacity for deposition and permeation through the skin.	[189]
Proniosomes	Coacervation phase separation	Cholesterol, span 60, lecithin	Brain delivery - migraine	Swiss albino mices' abdominal skin and albino rabbits' ear	Increased caffeine permeation through the skin and caffeine levels in blood and brain compared to orally administered caffeine. No evidence of skin irritation.	[181]
Nanoemulsion	Low energy emulsification	Dicaprylyl ether, ethylhexyl isononanoate, potassium lauroyl wheat amino acids,	Cosmetics, skin drug delivery	Abdominal human epidermis	Did not improve skin permeation of caffeine compared to emulsion.	[196]

		palm glycerides and capryloyl glycine				
Nanoemulsion	Low energy emulsification	Volpo-N10, oleic acid or eucaliptol	Skin drug delivery	human full-thickness skin	Increased permeation and retention of caffeine in hair follicles and skin.	[197]
Pickering emulsion stabilized by magnesium oxide NPs	High shear homogenization	Wheat german oil, magnesium oxide NPs	Oral drug delivery - hepatoprotective	Wistar rats intoxicated with CCl4	Decreased proliferation of cancer cells, moderate reduction of oxidative stress and inflammatory markers, similar to caffeine solution. Increased catalase levels compared to caffeine.	[198]
Polymer-based nanosystems						
Polymeric nanoparticles	Emulsion polimerization	Methyl methacrylate, CTAB or sodium dodecyl sulfate	antifungal	C. albicans	CTAB-caffeine nanoparticles inhibited the growth of *C. albicans*.	[199]
Polymeric nanoparticles	Desolvation	Gelatin	cancer	B16F10, L929 cell lines	Inhibited the proliferation of murine melanoma cells (B16F10) and induced apoptosis without causing cytotoxic effects on normal fibroblast cells (L929).	[193]
Metal-based nanosystems						
Silver complexes anchored to magnetic NPs	Covalent conjugation and complexation	Chloro-functionalized Fe3O4 magnetic NPs, caffeine N-heterocyclic carbene-silver complex	cancer	HepG2, WRL-68 cell lines	Enhanced cytotoxic effects against HepG2 cells and antibacterial activity against *E. coli*, *S. aureus* and *B. cereus*. Hyperthermia studies showed that the nanosystems reached a temperature of 47 °C, which is suitable for anticancer applications	[194]
Silver nanoparticles	Chemical reduction	Silver nitrate, galic acid, (-)-epicatechin-3-gallate or caffeine	cancer	B16-F0, COLO 679 cell lines	EGCG- and caffeine-stabilized AgNPs were the most and less effective against the tested cancer cell lines.	[200]
Gold nanoparticles	Chemical reduction	Gold (III) chloride trihydrate	antibacterial	*E. Coli, P. aeruginosa, S. aureus, L. monocytogenes*	Inhibition of biofilm formation and removal of mature biofilms. Antibacterial activity against resistant pathogenic bacteria.	[195]

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Crystal-based nanosystems						
Nanocrystals	Pearl-milling	Carbopol® 981, propylene glycol	skin drug delivery	Human volunteers, arm skin	The nanocrystals with a size of 694 nm showed a delayed but higher and longer delivery of caffeine, being detected in serum for at least 5 days	[201]

NLCs, nanostructured lipid carriers; AgNPs, silver nanoparticles; DPPG, 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol, sodium salt; CTAB, cetyltrimethylammonium bromide; EGCG, (-)-epicatechin-3-gallate;.

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6. Conclusions

In recent years, many studies have investigated the effects of caffeine on resistance, indicating that this area of research is growing rapidly.

Because toxic doses of caffeine are required to increase skeletal muscle contractility, binding of caffeine to adenosine receptors is likely the primary mechanism for the ergogenic effects of caffeine during endurance exercise.

The number of studies has increased considerably, and these studies provide new data on (i) the mechanisms of the ergogenic effects of caffeine endurance performance; (ii) the general acute and long-term effects of caffeine on resistance exercise; (iii) the effects of caffeine on women; (iv) the relationship between habitual caffeine consumption and the ergogenic effects of caffeine supplementation; (v) associations between genetic variations and individual responses to caffeine intake; (vi) optimal caffeine supplementation protocols (i.e., dose, source, and timing of caffeine); and (vii) placebo effects of caffeine during resistance exercise. However, it is also important to establish similar protocols to allow multiple comparisons between different sports and exercises.

However, this growing volume of articles, meta-analyses, and scientific evidence is not yet sufficient to confirm the quality and quantity of caffeine in sports. There are opportunities to explore in the future, such as: (1) comparing the effects of caffeine intake in different performance domains; (2) comparatively assessing the availability and strength of evidence for different performance domains; (3) making broad recommendations for the use of caffeine in specific sports and exercises, i.e., there are exercises where more than one motor skill predominates over others that are more isolated; and (4) making general recommendations for future research on the ergogenic effects of caffeine on athletic performance.

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