



Review

Lipid Signalling in Human Immune Response and Bone Remodelling under Microgravity

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Received: 28 May 2020; Accepted: 17 June 2020; Published: 23 June 2020



Abstract: Since the first Apollo mission in 1969, microgravity has been linked to many alterations of astronauts' physiology, among which immunosuppression, altered inflammation and bone loss represent relevant examples. In the past 40 years, extensive investigations have been conducted in order to characterize the molecular mechanisms driving the alterations caused by prolonged weightlessness on human health. However, almost all studies eluded the role played by bioactive lipids, a vastly heterogeneous class of endogenous molecules, which, under normal conditions, control immune and bone homeostasis. This is somewhat surprising, because it is widely accepted that pathological derangement of the production or signalling of these endogenous compounds leads to the onset and/or progression of numerous diseases. In particular, eicosanoids and endocannabinoids are known to play a role in immune responses and bone remodelling. Both classes represent the only lipids as yet investigated in Space, and are increasingly recognised as promising therapeutic candidates to combat different human disorders. This review summarizes evidence gathered in the past two decades on the changes in these two pivotal lipid signalling systems, through both simulated and authentic weightlessness (i.e., on board the International Space Station and in parabolic flights).

Keywords: microgravity; International Space Station; parabolic flight; inflammation; lymphocytes; bone loss; eicosanoid; endocannabinoid; cyclooxygenase; lipoxygenase

1. Microgravity in Human Health

The first evidence for the presence of pathophysiological alterations associated with microgravity was published in 1975, with the observation that 15 out of 29 of the Apollo mission's astronauts displayed increased viral or bacterial infections [1]. This observation, later confirmed by the analysis of blood samples of nine astronauts who participated in the Skylab missions [2], was finally explained by the studies of Dr. Cogoli's group, who demonstrated that Space flight induces lymphocyte immunosuppression [3–5]. Since then, many other investigations, conducted both through simulated and authentic microgravity, have demonstrated that exposure to Space conditions can trigger a plethora of physiological alterations, spanning from hemodynamic and cardiovascular complications [6], neuro-vestibular and circadian rhythm-related alterations, to loss of muscle tone and bone tissue [7,8]. These findings clearly demonstrated that understanding the molecular mechanisms behind these alterations, also in view of finding effective countermeasures to protect astronauts' health [9,10], represented an essential goal of the forthcoming prolonged Space missions, such as colonization of Mars.

Over the last 20 years, we have focused our attention on the role of lipid signalling on human immune response and bone remodelling under authentic microgravity conditions, taking advantage of four different missions. The first one took place in the year 2000 during the 28th parabolic flight campaign of the European Space Agency (ESA), with the goal of assessing the role of weightlessness on the catalytic constants of purified lipoxygenase-1 (LOX-1). Then, we performed the ROALD (Role Of Apoptosis in Lymphocyte Depression), RESLEM (Role of the Endocannabinoid System in human Lymphocytes Exposed to Microgravity), and SERiSM (Role of the Endocannabinoid System in Reprogramming Human Pluripotent Stem Cells under Microgravity) projects, which were all conducted on board the International Space Station (ISS), again to interrogate the possible impact of microgravity on *in vivo* lipid signalling. Against this background, here we sought to gather current knowledge on endogenous lipid signals in immune response and bone remodelling in Space, with a focus on the two most studied families of bioactive lipids: eicosanoids and endocannabinoids. Key studies performed on this topic by us and others are summarized in Figure 1, along with the microgravity conditions under which they were performed.

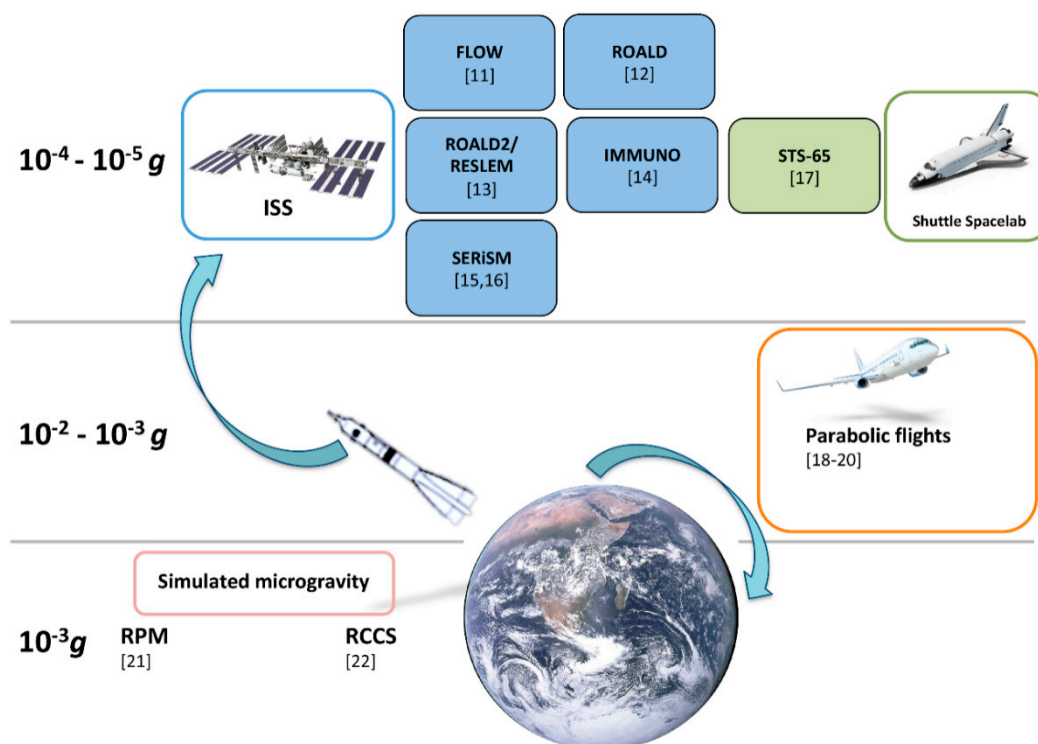


Figure 1. Experimental setup of the main studies that investigated endogenous bioactive lipids in microgravity [11–22]. RPM: random positioning machine; RCCS: rotary cell cultivation system.

1.1. Microgravity and Immune Response

A considerable body of investigations has demonstrated, in the past two decades, that microgravity has a significant impact on immune response, by acting on both innate and adaptive immunity, in a series of processes that can involve virtually all cells that participate in the inflammatory response (T lymphocytes, macrophages and endothelial cells), as well as the soluble molecular mediators released by them (such as cytokines, chemokines and, notably, bioactive lipids) [23,24]. Since the 1980s, CD4+ T cells have represented a main target of investigation: seminal studies conducted between the 1980s and the 1990s (reviewed in [25]) demonstrated that authentic microgravity, or simulated weightlessness in a random positioning machine (RPM) or rotary cell cultivation system (RCCS), can profoundly depress activation and proliferation of concanavalin A (ConA)- and CD3/CD28-stimulated T lymphocytes. These findings were also confirmed by additional studies,

which demonstrated that immune suppression is accompanied by dysfunctional cytokine signalling. Indeed, peripheral blood mononuclear cells (PBMCs) isolated from astronauts, collected and stimulated just after post-flight landing on Earth, showed a significant reduction in CD4 T cell-derived interferon (INF)- γ [26], while purified PBMCs that had been exposed to Space displayed suppression of interleukin (IL)-2 production and down-regulation of IL-2 receptor CD25 [4,5,27,28]. Remarkably, some alterations also became apparent under acute exposure to microgravity, and were recovered afterwards, as suggested by investigations showing that reduced INF- γ production from T cells was associated only with short duration flights (i.e., on the Space shuttle), while being absent in long-time missions (i.e., on board the ISS) [29,30].

Moreover, system biology-based meta-analysis of published data has recently shown that microgravity acts on the different cells that are involved in inflammation, by acting on a core signature of roughly 100 conserved signalling pathways [31]. In particular, repression of the tumour necrosis factor (TNF)/NF- κ B/Rel transduction axis, as well as of T-cell receptor (TCR)-mediated signals, was found in the transcriptomic, reactomic and miRNomic array datasets of a number of studies [32–35]. Moreover, the alteration of the adaptive immune response exerted by microgravity might have many implications, in that a recent work used a mouse *in vivo* model to demonstrate that Space flight impairs immune tolerance by significantly enhancing the production of inflammatory cytokines, such as INF- γ and IL-17, the latter molecule being engaged in the pathogenesis of a number of autoimmune conditions [36,37]. This effect seemingly depends on the depletion of T regulatory (Treg) cells through the repression of the IL-2/CD25 axis [37]. Spaceflight can also act on the processes that orchestrate inflammation by targeting other cellular components of the immune responses, such as innate immune processes that orchestrate inflammation cells, as well as on vascular structures. Indeed, microgravity impairs macrophage differentiation and overall polarization [38] and alters complement system activation [39], while eliciting metabolic reprogramming and altering cytokine production [38,40]. Recent studies have also reported a direct effect on endothelial cells, which consisted of the down-regulation of adhesion molecules that regulate the recruitment of immunocytes at the inflamed site [41], as well as of the enhancement of the Nlrp3-dependent inflammatory cascade [42]. Of note, microgravity might also affect inflammatory signalling in immune cells by acting on membrane homeostasis: indeed, membrane fluidity plays a role in cell physiology, and many soluble mediators that modulate immune functions can exert their effect through changes in membrane dynamics [43]. Furthermore, membrane fluidity can affect *per se* immune cell functions by acting on T cell receptor (TCR) [44] and Toll-like receptor (TLR) signalling [45], as well as by controlling antigen presentation [46]—mostly by acting on the distribution and function of lipid rafts—and thus the immune response. In this context, microgravity can change membrane fluidity [47], suggesting that the Space environment might also affect lipid raft-mediated signalling in immune cells, which in turn might affect the production of pro-inflammatory mediators, bioactive lipids included. An additional point of action might be represented by the impairment of hematopoietic tissues, and of the maturation of immune cells: indeed, microgravity can induce atrophy of both bone marrow and thymus, which control generation and maturation of T and B cells [48]. Even though several groups recently investigated the molecular mechanisms behind Space flight-induced immune alterations, the full array of molecular actors behind them remains mostly elusive, with the biggest part of the published literature having focused on cytokines. Instead, the role of endogenous bioactive lipids, which are produced by the cells that orchestrate inflammation [49], has been poorly addressed. The latter molecules represent the fulcrum of the immune response, governing the initiation, extent and outcome of the inflammatory event, and understanding their role in the modifications elicited by microgravity on human immune networks represents a crucial piece of the puzzle that will ultimately allow safe Space travel in the near future.

1.2. Microgravity and Bone Remodelling

Besides inflammatory response, bone homeostasis represents the main issue in Space-related pathophysiology, due to the fact that prolonged Space flights lead to decreased bone deposition and osteoporosis [50].

A dynamic bone remodelling results from the balance between deposition and resorption of hydroxyapatite mineral matrix, regulated by bone-resident osteoblasts and osteoclasts, respectively. This balance is regulated both remotely through the parathormone/calcytonine axis, which modulates osteoblasts and osteoclasts, and locally through osteocytes embedded in the mineral matrix that act as mechanosensors, translating the compressive forces exerted by gravity on the tissue into biological and hormone-like signals that participate in bone homeostasis [51].

In particular, continuous mechanical stimulation is required for proper osteocyte function, as demonstrated by the fact that lack of mobility (achieved through surgical intervention on animal models) and authentic microgravity both induce osteocyte apoptosis and the recruitment of osteoclasts that resorb bone matrix [52–55]. Microgravity can also directly hinder bone mineralization, by inhibiting activation and differentiation of osteoblasts from their progenitors [56–59], as well as by directly inducing osteoclastogenesis [58,60]. To date, the molecular mechanisms behind this process have not yet been fully disclosed, however it is thought that cytoskeletal proteins can sense gravity, the lack of which triggers detrimental signals in bone cells. An involvement of bioactive lipids in these processes is quite expected, because all cells involved in bone homeostasis can produce and/or respond to eicosanoids [61,62], endocannabinoids [63,64] and glycerophospholipids [65]. Yet, investigations into lipid signalling in bone remodelling are still scarce.

2. Bioactive Lipids in Inflammation and Bone Remodelling

Endogenous bioactive lipids can be grouped into five main classes, which include widely investigated molecules like eicosanoids and endocannabinoids, as well as other compounds such as specialized pro-resolving mediators (SPMs), sphingolipids and glycerophospholipids. These substances are produced by virtually all cells of the body and, during phlogistic events, they control recruitment, removal and turn over of the immune cells, as well as their activation, differentiation and systemic trafficking [49], thus being involved in several—if not all—pathophysiological processes that lay at the base of inflammatory and immune diseases [49]. To date, data are available only on the role of eicosanoids and endocannabinoids in microgravity-induced alterations of immune response and bone remodelling. Thus, their chemistry, signalling and pathophysiology will be summarized herein, whereas that of the other lipid congeners—i.e., SPMs, sphingolipids and glycerophospholipids—can be found in recent, extensive reviews [66–68].

2.1. Eicosanoids: Chemistry, Signalling and Pathophysiology

Eicosanoids are the endogenous lipids that have been studied most thoroughly and for the longest time. They are produced during the whole inflammatory event, where they sustain the activation of the immune system during both acute and chronic inflammation. In addition, eicosanoids control a number of non-pathological processes, such as gastroprotection and kidney blood flow, the impairment of which is at the basis of the undesired side-effects of prolonged use of non-steroidal anti-inflammatory drugs [69]. The synthesis of eicosanoids starts when arachidonic acid (AA) is cleaved from the *sn*-2 position of membrane phospholipids, through the action of phospholipase A₂ (PLA₂), and then is channeled towards distinct biosynthetic routes: the cyclooxygenase-1/2 (COX-1/2)-dependent dioxygenation of AA conveys prostaglandin H₂ (PGH₂), which in turn is converted into 4 prostaglandins (i.e., PGI₂/D₂/E₂/F_{2α}) or into thromboxane A₂ (TXA₂), by means of their respective synthases [70]. The other main branch of the so called “AA cascade” goes through the catalytic action of 5-, 12- and 15-lipoxygenase (5-, 12- and 15-LOX) isozymes, and yields different leukotrienes (LTs) [71], along with other minor congeners, such as the hydroperoxides (HpETE) and hydroxides (HETE) of

AA, and lipoxins [71,72]. Eicosanoids act by engaging distinct G protein-coupled receptors (GPCR), as summarized in Figure 2, and act as the main scaffold of the inflammatory network during the immune response [49]. Indeed, they are produced by (and act on) immune cells during the onset of acute and chronic inflammation, when they enhance the response to pathogen- and damage-associated molecular patterns (PAMPs and DAMPs, respectively) and to cytokines, and promote the differentiation, recruitment and activation of pro-inflammatory lymphocytes (e.g., T helper (T_H)1 and T_H17) and monocytes/macrophages [49,73]. Eicosanoids have also been involved in bone tissue remodelling. In particular, PGE₂ seems to be mainly involved in bone turnover, where it promotes both matrix deposition and resorption, possibly by acting on different receptors of osteoblasts and osteoclasts, in concert with other hormonal signals [61,62]. The main metabolic pathways leading to eicosanoid synthesis, and the main targets on which microgravity is known to act, are summarised in Figure 2.

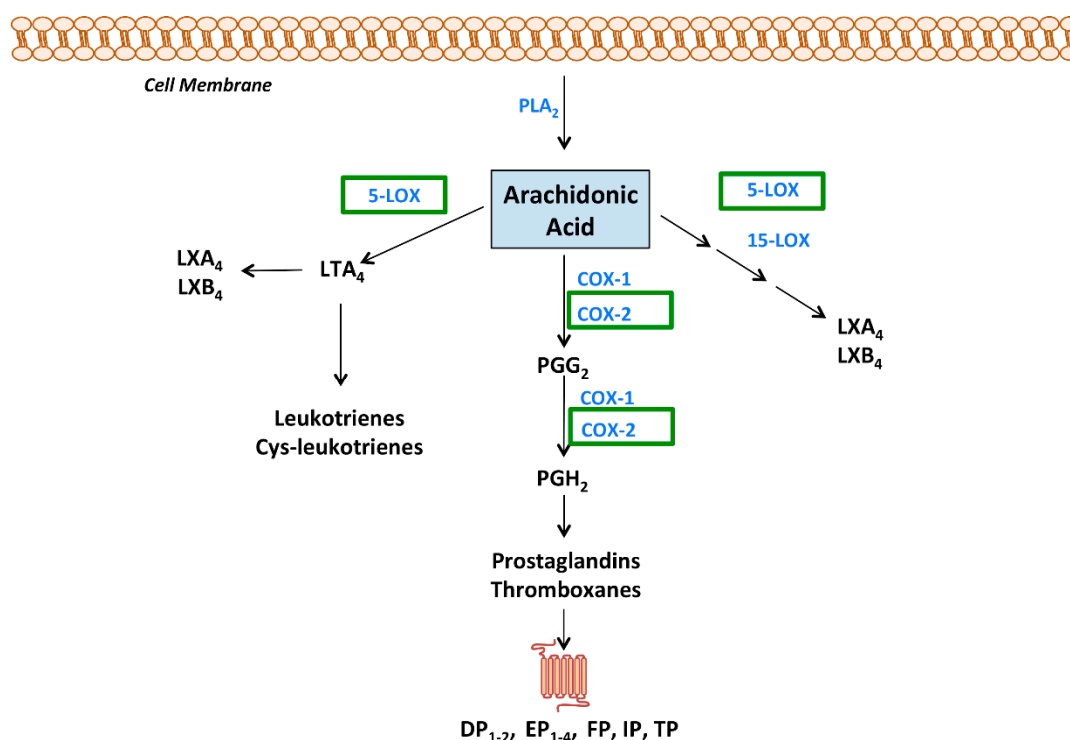


Figure 2. Biosynthetic pathways of the main arachidonic acid (AA)-derived bioactive lipids. Targets that are up-regulated in microgravity are highlighted in green. COX: cyclooxygenase; CysLT: cysteinyl leukotriene; DP: prostaglandin D receptor; EP: prostaglandin E receptor; FP: prostaglandin F receptor; IP: prostaglandin I receptor; LOX: lipoxygenase; LT: leukotriene; LX: lipoxin; PG: prostaglandin; PLA: phospholipase A; TP: thromboxane receptor.

2.2. Endocannabinoids: Chemistry, Signalling and Pathophysiology

Endocannabinoids (ECs) are endogenous lipids that engage the same cell receptors targeted by the main psychoactive ingredient of *Cannabis sativa* and *Cannabis indica*, namely Δ^9 -tetrahydrocannabinol (THC) [74]. By binding to type-1 and type-2 (CB₁ and CB₂) cannabinoid receptors (that belong to the GPCR family), and to transient receptor potential vanilloid 1 (TRPV1) channels, ECs are involved in several pathophysiological processes, acting both as neuromodulators [75,76] and immunomodulators [77]. *N*-Arachidonylethanolamine (AEA, also known as anandamide) and 2-arachidonoylglycerol (2-AG) are the two best studied ECs, and with their biosynthetic—i.e., *N*-acylphosphatidylethanolamines-specific phospholipase D (NAPE-PLD) and diacylglycerol lipase (DAGL), for AEA and 2-AG respectively—and breakdown enzymes—i.e., fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), for AEA and 2-AG respectively—they form the so-called EC system [77].

AEA biosynthesis starts with the *N*-acylation of membrane phosphatidylethanolamine, catalysed by *N*-acyltransferase (NAT), to yield *N*-arachidonoyl-phosphatidylethanolamine (NArPE), which is then hydrolysed to AEA by NAPE-PLD [78]. Instead, degradation of AEA is catalysed by FAAH, which cleaves AEA into AA and ethanolamine [77].

As for 2-AG, phospholipase (PL)C-catalysed hydrolysis of membrane phospholipids yields diacylglycerol, which is further hydrolysed by DAGL to produce 2-AG [77]; this is then cleaved into AA and glycerol by MAGL [79] and, to a lesser extent, by FAAH [80]. ECs can target several immune cells: AEA is able to suppress the pro-inflammatory activation and cytokine secretion in human lymphocytes [81] and dendritic cells [82], while 2-AG is able to enhance macrophage phagocytosis [83]. Their involvement in the control of inflammation is also suggested by several studies that point to EC-related dysfunctions as a cause of inflammatory pathologies, such as multiple sclerosis or inflammatory bowel diseases [49,74,84–86]. The main EC system elements are summarised in Figure 3.

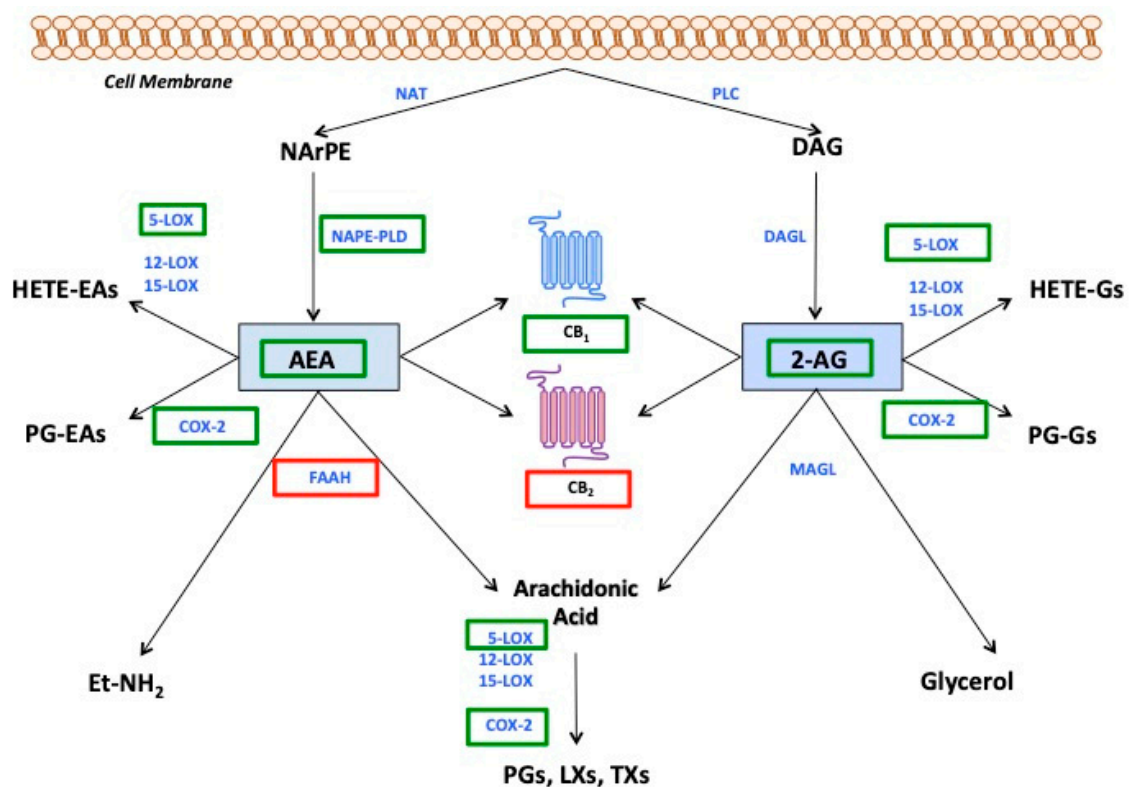


Figure 3. Metabolism of endocannabinoids and related bioactive lipids. Targets that are up-regulated or down-regulated in microgravity are highlighted in green or red, respectively. 2-AG: 2-Arachidonoylglycerol; AEA: *N*-Arachidonylethanolamine; CB: Cannabinoid receptor; DAG: Diacylglycerol; DAGL: Diacylglycerol lipase; EtNH₂: Ethanolamine; FAAH: Fatty acid amide hydrolase; HETE-EA: Hydroxyeicosatetraenoyl-ethanolamine; HETE-G: Hydroxyeicosatetraenoyl-glycerol; LOX: Lipoxygenase; LX: lipoxin; MAGL: Monoacylglycerol lipase; NAPE-PLD: *N*-Acyl phosphatidylethanolamine specific-phospholipase D; NAT: *N*-Acyl transferase; NArPE: *N*-Arachidonoyl-phosphatidylethanolamine; PA: Phosphatidic acid; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PG: Prostaglandin; PI: Phosphatidylinositol; PLC: Phospholipase C; TX: Tromboxane.

3. Lipid Signalling in Microgravity

3.1. Eicosanoids

The possibility that metabolic enzymes of eicosanoids could be altered by microgravity was documented for the first time by us with a project performed under authentic microgravity during the 28th parabolic flight campaign of ESA [21]. These experiments measured the catalytic constants

(Michaelis–Menten constant, K_m , and maximum velocity, V_{max}) of a purified LOX-1, using the EMEC (Effect of Microgravity on Enzymatic Catalysis) module, a fiber optics spectrophotometer specifically developed to measure enzyme activity in weightlessness [21]. Microgravity ($\sim 10^{-2} g$) was shown to reduce the K_m value (i.e., to enhance the affinity) of LOX-1 for its fatty acid substrate without affecting V_{max} , thus pointing to LOX-1 as the first protein able to act as a direct “gravity sensor” [18].

Later on, we reported that simulated microgravity ($\sim 10^{-3} g$)—achieved by means of clinorotation—is able to almost double COX-2 activity in human bone marrow K562 cells, whereas simulated hypergravity had the opposite effect [21], further supporting the observation that eicosanoid-metabolising enzymes do react to gravitational changes. Remarkably, COXs and LOXs represent the two main enzymes controlling eicosanoid levels during inflammation, therefore these data suggest that microgravity might have an impact on phlogistic processes by targeting AA-derived endogenous lipids. In addition, LOXs are well-known modulators of immune cell biology [49], and their alteration during spaceflight might represent one of the causes leading to the microgravity-related impairment of lymphocyte function. We were able to confirm this hypothesis in two additional projects. In the first study, we demonstrated that human peripheral blood mononuclear cells (PBMCs) undergo enhanced apoptosis when exposed to RPM-simulated microgravity ($\sim 10^{-3} g$) and—most importantly—that programmed cell death was strictly due to enhanced 5-LOX activity. Indeed, microgravity-induced apoptosis was prevented by 5-LOX pharmacological inhibition, and human U937 immune cells devoid of 5-LOX were resistant to it [87]. Of note, the engagement of 5-LOX in this type of apoptosis was isozyme-specific, as pharmacological inhibition of other LOX isoforms had no effect [87]. More recently, we were also able to extend these data under authentic microgravity, by studying human PBMCs on board the ISS in the frame of the ROALD mission [68]. During this project, not only could we confirm that human PBMCs display enhanced apoptosis-associated markers (e.g., DNA fragmentation and poly-ADP ribose polymerase expression), but also that this feature was due to increased 5-LOX activity [12]. Additional studies corroborated and extended these findings, showing that 5-LOX inhibition reverts both apoptosis and altered production of pro-inflammatory cytokines (e.g., IL-2 and INF- γ) in human Jurkat T cells [22].

Besides the alterations of immune functions, bone loss is a major threat to astronauts’ health during Space missions, and represents a limit to long-term manned flight. Eicosanoids are known to be involved in the homeostasis of bone tissue [88–90], however, to date, only a few data are available describing the alteration of their signalling pathways or metabolism upon prolonged Space flights. In particular, microgravity has been shown to trigger up-regulation of COX-2 and enhanced production of PGE₂, as well as increased production of IL-6 in osteoblasts [11,17,91,92].

3.2. Endocannabinoids

ECs and the complex EC system have also attracted considerable interest in microgravity-related pathophysiology, especially in the light of their nature as versatile agents involved in adaptation to various environmental and metabolic conditions, immune response and bone remodelling included [14,77]. During the last two decades, a number of projects have provided insight into the effect of authentic or simulated microgravity on EC signalling and metabolism, as well as on the possible implications of this effect for human health in Space.

In particular, we performed two independent missions on board the ISS, namely RESLEM (which was part of the 2011 PromISSE mission organized by ESA), and SERISM (which was performed in 2017 in the frame of the Italian Space Agency VITA mission). These were among the few studies proving that the EC system is indeed one of the targets through which weightlessness triggers immune alterations and loss of bone density [13,19,20,93].

The data obtained from the RESLEM experiment showed that, under authentic microgravity, human PBMCs undergo a time-dependent re-modulation of the two main AEA metabolic enzymes. After 48 h on the ISS, a down-regulation of FAAH and a concomitant up-regulation of NAPE-PLD

were observed, both at mRNA and protein levels, suggestive of enhanced production of AEA as a likely adaptive response of immune cells to prolonged microgravity [13].

Incidentally, LOXs can also control endogenous AEA content by producing AEA hydroperoxides that competitively inhibit FAAH [12]. As reported above, microgravity also induces an early increase of 5-LOX activity in PBMCs (and hence an inhibition of FAAH), further supporting the concept of increased AEA content as a crucial response and adaptation of PBMCs to microgravity [12]. Interestingly, the effect of microgravity on EC production and immune response is also supported by other recent studies, such as the one stemming from the IMMUNO mission, which assessed the immune functions and stress levels of ISS crew members undergoing long-term Space flight [77]. This project confirmed a significant and persistent increase of EC levels (especially AEA) in blood, also associated with altered immune cell repertoire and functionality characterized by enhanced numbers of neutrophils, NK cells, B cells and monocytes, reduced amounts of Treg lymphocytes, as well as by an overproduction of pro-inflammatory cytokines (e.g., TNF and IL-1 β) and a reduction of the anti-inflammatory ones (TGF- β , and IL-10) [14].

Interestingly, increased EC blood levels were also reported by other studies, where microgravity was achieved through parabolic flights [19,20]. In this paradigm, human subjects were exposed to low-duration (~ 20 s) weightlessness, suggesting that ECs are not only involved in long-term adaptation to Space—where they might act on processes like immune response independently of stress sources—but also as stress-induced adaptive agents that participate in responding to immediate environmental modifications. Interestingly, only individuals who did not experience flight-related motion sickness showed enhanced EC levels, while those experiencing kinetosis did not show any significant change [19,20]. Again, these findings point to a role of ECs in the swift adaptation to altered gravity.

It should be recalled that ECs are also involved in bone metabolism [63,64]. This issue was addressed by the SERiSM project, whereby human blood-derived stem cells (hBDSCs) were driven towards osteogenic differentiation under authentic microgravity on board the ISS [15,93]. In this cellular model, possible alterations of the EC system were also interrogated [16,93]. Our study demonstrated that microgravity modulates the expression of the two main cannabinoid receptors of hBDSCs, eliciting an up-regulation of CB₁ and a down-regulation of CB₂ [80]. Since CB₂ is known to induce mitogenic signals in osteoblasts [94,95], and CB₁ and CB₂ stimulate osteoclast formation by promoting bone resorption [96–98], these data suggest that prolonged weightlessness might unbalance EC signalling towards loss of bone mass. Incidentally, neither the other EC-binding receptor TRPV1, nor any of the EC metabolic enzymes (NAPE-PLD, FAAH, DAGL and MAGL) of hBDSCs were affected by microgravity [16].

4. Conclusions and Future Perspectives

Bioactive lipids control one of the most intricate signalling networks as yet known, and lay at the basis of most of the known pathophysiological processes. However, their possible alteration under Space conditions has been poorly investigated. Available data, summarized in Table 1, strongly suggest that these molecules also contribute to the effects of microgravity on human immune response and bone remodelling, which are known to culminate in the pathological alterations caused by prolonged spaceflights in astronauts. The studies that addressed these issues, though still relatively scarce, are increasingly pointing to eicosanoids and endocannabinoids as promising molecular targets, both in evaluating future countermeasures for astronauts and in fully elucidating the pathogenesis of Space-associated disorders. Moreover, the body of information gathered so far has not yet interrogated other relevant lipids that are deeply embedded in the mesh of cellular and molecular processes that control inflammation and bone remodelling (e.g., SPMs, sphingolipids and glycerophospholipids), which remain to be clarified. In this context, an involvement of SPMs, which spatially and temporally confine inflammation, might represent a promising target in Space-related bio-medicine, especially in the light of the fact that many symptoms displayed by the astronauts are

indeed suggestive of an unbalanced immune response, and hence are potentially linked to many pathological paradigms [97–100]. For instance, the well-documented effect of microgravity on LOXs represents a promising hint, inasmuch as 5-LOX, 12-LOX and 15-LOX not only govern the eicosanoid tone, but are also the biosynthetic machinery for the synthesis of all known SPMs. Thus, chances are that microgravity may also act on SPM signalling and metabolism in a rather pleiotropic manner. Future studies will tell whether targeting bioactive lipid signalling and metabolism will turn out to be a successful strategy for the development of effective and well-tolerated countermeasures (and eventually therapies), able to preserve astronauts' health during prolonged Space missions.

Table 1. Cell systems, molecular targets and effects of microgravity.

Sample	Target	Effect of Microgravity	Experimental Setup	Reference
PBMCs	AEA	Enhanced production	ISS	[13]
Human Blood	AEA	Enhanced production	ISS	[14]
Human Blood	AEA	Enhanced production	Parabolic flight	[19,20]
PBMCs	FAAH	Down-regulation (both at transcriptional and protein level)	ISS	[13]
PBMCs	NAPE-PLD	Up-regulation (both at transcriptional and protein level)	ISS	[13]
Osteoblasts	PGE ₂	Enhanced production	Shuttle Spacelab	[17]
hBDSCs	CB ₁	Enhanced production	ISS	[16,93]
hBDSCs	CB ₂	Lower production	ISS	[16,93]
Purified enzyme	LOX-1	Enhanced activity	Parabolic flight	[18]
K562 cells	COX-2	Enhanced activity	RPM	[21]
Lymphocytes, U937 cells	5-LOX	Enhanced activity	RPM	[12,87]
PBMCs	5-LOX	Enhanced activity	ISS	[12]
Jurkat T cells	5-LOX	Enhanced activity	RCCS	[22]

Author Contributions: All authors listed have made a substantial, direct and intellectual contribution to the work and approved it for publication. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: We wish to express our thanks for the financial support over the last 20 years from the Italian Space Agency (ASI), European Space Agency (ESA) and National Aeronautics and Space Agency (NASA), which made possible our studies on bioactive lipids in microgravity. In addition, the fruitful collaboration with Kayser Italia S.r.l. (Livorno, Italy), that developed the hardware of ROALD, RESLEM and SERISM, is gratefully acknowledged.

Conflicts of Interest: The authors declare no conflict of interest.

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