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Iron citrate Synthesit: Impact on blood parameters in non-iron deficient models

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Abstract: Iron's significance extends beyond treating anemia, impacting various disorders. However, studies on iron-fortified diets in healthy subjects are limited. To evaluate the effects of Synthesit iron citrate on biochemical, hematological parameters, and body weight in aged rhesus macaques, and its safety in SHK-line mice. An experimental study involved SHK mice and rhesus macaques receiving Synthesit iron citrate, with blood parameters, histology, hematopoietic stem cells, and biochemistry monitored over 30 days (14 mice) and six weeks (8 macaques). The study shows increased hematopoietic cell types and mitosis index, with significant increases in Myelokaryocyte count (32.4%), megakaryocyte count (3.7-fold), myeloblast count (1.7-fold), mononuclear cell count (2.5-fold), erythroblast count (4-fold), and reticulocytes (13.3%). In macaques, supplementation initially reduced erythrocyte count, followed by recovery, while thrombocyte count increased initially but decreased post-supplementation. Leukocyte analysis showed varied responses, with a decrease in monocyte and lymphocyte but an increase in neutrophil percentage. Biochemical analyses revealed improvements in glucose (baseline: 4.36 ± 0.32 mmol/l vs day 43: 3.30 ± 1.06 mmol/l), cholesterol (baseline: 4.563 ± 0.799 mmol/l vs day 29: 2.84 ± 0.88 mmol/l), and triglyceride levels (baseline: 1.49 ± 0.63 mmol/l vs day 29: 0.70 ± 0.34 mmol/l). The novel study reveals the effects of iron citrate synthesis in non-iron deficient models into the blood parameters which has metabolic potential. In conclusion, Synthesit® iron citrate supplementation exhibits promising effects on hematopoiesis and metabolic parameters in murine and primate models.

Keywords: *Cholesterol, AST, Hematopoietic stem cells hematopoiesis, Iron citrate supplementation.*

1. Introduction

Iron is not just important for treating anemia; it also plays a critical role in several bodily functions. It is essential for various proteins and enzymes involved in oxygen transport, cellular respiration, DNA SYNTHESIT, and gene regulation [1]. While iron supplements are effective for managing anemia, taking excessive doses exceeding 45 mg per day can lead to digestive issues. Studies have shown that up to 60% of individuals taking oral iron supplements report gastrointestinal side effects [2,3]. Iron replacement therapy has been found to have several benefits. It can help normalize hemoglobin concentration, restore work capacity, and alleviate fatigue in individuals with iron deficiency anemia [4,5].

Dietary supplements provide various iron sources, commonly in the form of either ferrous or ferric salts. For instance, ferrous sulfate or gluconate, and ferric citrate or sulfate are commonly used [6]. Ferrous ionic forms generally offer higher bioavailability compared to ferric ionic forms in salts [7]. However, doses of iron supplementation exceeding 45 mg/day may lead to gastrointestinal side effects

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such as constipation, nausea, and diarrhea, rendering them intolerable for patients. On the other hand, there are alternative forms of iron supplementation available, which are generally better tolerated. These include heme-based iron polypeptides, carbonyl iron, iron amino-acid chelates, and polysaccharide-iron complexes [8]

Iron bioavailability, a crucial aspect of nutrient absorption in the body, is subject to various factors and can be enhanced through strategic dietary interventions. The presence of ascorbic acid, commonly known as vitamin C, has been observed to positively impact the absorption of nonheme iron. This effect is dose-dependent and is most pronounced when both nutrients are consumed together [9,10]. Animal-derived foods, such as beef, chicken, fish, pork, and lamb, have also been identified to facilitate the absorption of dietary non-heme iron [11]. The standard definition of intestinal nutrient bioavailability refers to the proportion of nutrients from ingested food that are absorbed and utilized by intestinal erythrocyte cells [12]. Several factors influence iron bioavailability, including the form of iron (heme and nonheme), dietary inhibitors such as calcium and phytates, and enhancers like ascorbic acid and proteins. Different food types exhibit varying rates of iron absorption. For instance, consumption of organ meats can lead to a 25-30% absorption rate, while green leafy vegetables, cereals, and dried legumes have absorption rates of 7-9%, 4%, and 2%, respectively. Studies indicate that interactions between nutrients play a significant role in dietary iron absorption, with vitamin C notably enhancing iron absorption. Moreover, the bioavailability of iron can be influenced by food processing techniques. For instance, in the case of bread, certain fermentation methods, such as microencapsulation of iron coated with whey protein isolate and a starch-based aqueous coating, have been shown to increase cellular absorption by up to 73% [10].

Iron deficiency anemia has been shown to have detrimental effects on cognitive development in children, as documented in references [13]. Conversely, an excessive accumulation of iron in the body can lead to bone marrow failure and dysfunction in parenchymal organs, as indicated by previous studies. Furthermore, it has been observed that maternal iron deficiency anemia is associated with an increased risk of low birth weight and preterm delivery, as outlined in reference [14].

Iron deficiency is a prevalent global health issue that often leads to anemia. Treatment with iron replacement therapies not only boosts hemoglobin levels but also impacts other blood parameters, including thrombocyte count. Some cases have shown a decrease in thrombocyte count during iron replacement therapy, although this thrombocytopenia typically resolves on its own [15].Both insufficient and excessive iron levels pose significant health risks. Iron deficiency can lead to growth arrest and anemia, while iron overload, often caused by factors like frequent blood transfusions or excessive gastrointestinal absorption, can result in tissue damage and organ dysfunction. Excess iron triggers oxidative stress, generating harmful radicals and causing tissue damage. Ferroptosis, a form of cell death dependent on iron, involves lipid peroxidation-induced membrane damage and controlled necrosis [16].Iron overload leads to the accumulation of free iron in the body, surpassing transferrin's binding capacity and forming "non-transferrin-bound iron" including labile plasma iron. LPI, being reactive and capable of binding to other molecules, can enter cells through voltage-gated calcium channels, particularly affecting cardiac muscle cells. The connection between iron and cardiovascular diseases has been extensively studied, although evidence supporting this link is not consistently strong due to variations in iron markers used across studies. The role of iron in CVD remains a topic of ongoing debate [17].Initially considered a cardiovascular risk factor, the relationship between iron and conditions like atherosclerosis and coronary artery disease is complex. Early hypotheses suggested iron's involvement in oxidizing LDL cholesterol and contributing to arterial plaque formation [18].

When assessing the efficacy of iron supplements, it is common practice to prioritize the monitoring of hemoglobin concentration. In addition to hemoglobin, secondary indicators such as ferritin, red cell distribution width, mean corpuscular volume, soluble transferrin receptor, total iron binding capacity, serum iron, and transferrin saturation are often considered as part of the evaluation process, as highlighted in reference [19]. Recent studies have highlighted a possible association between dietary iron intake and the development of certain health conditions such as type 2 diabetes and cholesterol

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levels. For instance, a prospective cohort study indicated that the consumption of heme iron found in red meat may increase the risk of developing type 2 diabetes [20]. Similarly, a survey conducted in China suggested that both heme and non-heme iron intake could potentially elevate the risk of type 2 diabetes [21].

Furthermore, experiments conducted on rats demonstrated that low-iron diets led to improvements in lipid profiles and glucose tolerance, suggesting potential metabolic benefits [22]. In terms of addressing iron deficiency, iron-fortified foods have emerged as a cost-effective supplementation method. However, they encounter challenges such as alterations in taste. Oral iron supplements, like ferrous sulfate, are commonly used but can lead to gastrointestinal discomfort [23]. Oral administration of iron supplements is a prevalent choice for delivering iron due to its affordability, convenience, and independence from external conditions [24]. Ferrous sulfate tablets, often regarded as the archetype of oral iron supplements, are both easy to handle and cost-effective, marking them as first-generation options [25]. Emerging alternatives such as ferrous citrate are designed to tackle absorption challenges, yet they may still induce gastrointestinal reactions. There is growing interest in investigating the potential of low iron concentrations in managing metabolic disorders [26]. The research aims to evaluate Synthesit iron citrate effects on rhesus macaques' biochemical and hematological parameters, body weight, and on hematopoietic stem cells in mice. This exploration seeks to understand the supplement's impact on health markers in these animal models, potentially shedding light on its implications for human health.

1.1. Aims and Objectives

1. Evaluate the impact of iron citrate Synthesit on biochemical and hematological parameters in aged rhesus macaque primates.
2. Analyze changes in body weight among experimental animals following iron citrate Synthesit supplementation.
3. Investigate the safety and efficacy of long-term oral administration of Synthesit iron citrate on SHK-line laboratory mice.
4. Provide insights into the potential implications of iron supplementation for human health.

2. Methodology

2.1. Study Design

This study employed an experimental method involving animal models and in vitro assays. Two distinct animal models were utilized SHK line mice and male rhesus macaques.

2.2. Study Setting

Study was conducted at the Research Institute of Medical Primatology in Sochi, Russia.

2.3. Sample Size and Sampling Technique

For the SHK line mice experiments, a total of 14 mice were utilized, divided equally into two groups. In the macaca mulatta experiments, eight male rhesus macaques were included. In vitro studies were conducted using Thp1 monocytic cells and human primary fibroblasts cultured in a controlled CO2 incubator

2.4. Material

Synthesit iron citrate was the primary material used for supplementation in both animal models and in vitro assays. The supplement, commercially available from Scientific Research Center Synthestech. Additionally, standard laboratory materials and reagents were employed for tissue processing, staining, biochemical analysis, and cell culture

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2.5. Data Collection

Following procedure was adopted to collect information

1. For SHK Line Mouse Experiments, fourteen SHK line mice were utilized, divided into two groups: one receiving Synthesit iron citrate and the other serving as the control. Over a period of 30 days, their consumption was monitored, and upon euthanasia following AVMA Guidelines, various tissues including the heart, liver, lungs, spleen, brain, kidneys, testes, and bone marrow were collected for histological examination. Bone marrow was extracted from the femur using standard procedures. Tissue samples were then processed, embedded in paraffin, and stained with hematoxylin-eosin. Additionally, bone marrow smears were stained using the May-Grünwald-Giemsa method for microscopic analysis. Morphological evaluation was conducted using a microscope from Carl Zeiss Microscopy GmbH, Germany, with bone marrow mononuclear cells being manually counted.
2. For Macaca Mulatta Experiments, eight male rhesus macaques received daily oral doses of Synthesit iron citrate for 46 days. Hematological measurements were taken on days 0, 18, 32, and 46, while biochemical parameters were assessed on days 0, 15-, 29-, 43-, and one-week post-supplementation (day 50). Throughout the study, body weight and food intake were monitored at specific intervals. The impact on body weight was evaluated using weekly electronic scale measurements. Blood samples were collected and centrifuged at 3500 rpm to obtain serum for biochemical analysis. Hematological analysis, including parameters such as hemoglobin, erythrocyte count, leukocyte count, and percentages of lymphocytes, monocytes, neutrophils, eosinophils, and basophils, was performed using the Bechman 5 diff hematological analyzer following the manufacturer's recommendations.
3. In Vitro Studies, Thp1 monocytic cells and human primary fibroblasts were cultured in a CO2 incubator in DMEM medium supplemented with fetal bovine serum and gentamicin. Human fibroblasts were exposed to Synthesit iron citrate for 72 hours, followed by the MTT assay to assess viability. Thp1 monocytic cells were treated with Synthesit iron citrate for 24 hours. RNA extraction was carried out using the ExtractRNA kit, and its concentration and quality were evaluated using a NanoDrop spectrophotometer. Reverse transcription was performed with MMLV-RT, followed by Real-time PCR using qPCRmix-HS SYBR reagent mix on a BioRad Connect™ Real-Time PCR Detection System. Gene expression analysis included IL6, IL1B, and CCL2 genes.

2.6. Statistical Analysis

Statistical analysis was performed using various software including Statistics 6.0, Microsoft Excel 2010, and GraphPad Prism. For macaques, one-way ANOVA tests were employed to assess differences between administration time points ($p < 0.05$). For mice, statistical significance between groups was determined using the Mann-Whitney U test ($p = 0.05$) due to small sample sizes. Data were presented as mean ± SD for descriptive analysis.

2.7. Ethical Approval

All procedures involving animals were conducted in strict accordance with ethical guidelines, including the Declaration of Helsinki and the European Convention for the protection of vertebrate animals' welfare used for experimental and other scientific purposes document. The study was approved by the Bioethics Commission, ensuring compliance with universally recognized scientific, ethical, and legal standards.

3. Results

Table 1 shows that after one month of treatment, murine bone marrow and organ tissues were collected for histological examination. Hematopoietic stem cell analysis revealed a notable increase in the mitosis index, which was twice as high in the experimental group compared to the control group

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(0.2 vs. 0.1). Additionally, the experimental group exhibited significant increases in various hematopoietic cell types. Specifically, Myelokaryocyte count was 32.4% higher in the experimental group (22.2 ± 1.2 x 106) compared to the control group (15.0 ± 0.9 x 106). Moreover, there was a 3.7-fold increase in megakaryocyte count (1.1 ± 0.1 x 106 in the experimental group vs. 0.3 ± 0.0 x 106 in the control group), indicating activation of thrombopoiesis. Monopoiesis was also enhanced, with myeloblast and mononuclear cell counts being 1.7-fold and 2.5-fold higher in the experimental group, respectively. Furthermore, erythroblast count was nearly four times higher in the experimental group compared to the control group (1.9 ± 0.1 x 106 vs. 0.5 ± 0.1 x 106), with a modest increase in reticulocytes observed as well (13.3%). Importantly, the count of hematopoietic stem cells remained within the normal physiological range. These findings suggest that oral supplementation with Synthesit iron citrate stimulates various types of hematopoiesis in murine bone marrow.

Table 1.
Effect of synthesit iron citrate oral supplementation on hematopoiesis in murine bone marrow.

Parameters	Control group	Experimental group	Difference
Total Myelokaryocyte count (Per hip) x 10^6	15.0 ± 0.9	22.2 ± 1.2*	32.4%
Reticulocytes x 10^6	1.3 ± 0.1	1.5 ± 0.1*	13.3%
Undifferentiated blasts (Mononuclear cells) x 10^6	2.2 ± 0.1	5.4 ± 0.1*	59.3%
Myeloblasts x 10^6	2.1 ± 0.1	3.5 ± 0.1*	40%
Mitotic index	0.1 ± 0.0	0.2 ± 0.0*	50%
Lymphocytes x 10^6	16.8 ± 1.8	22.0 ± 1.1*	23.6%
Megakaryocytes x 10^6	0.3 ± 0.0	1.1 ± 0.1*	72.7%
Erythroblasts x 10^6	0.5 ± 0.1	1.9 ± 0.1*	73.68%

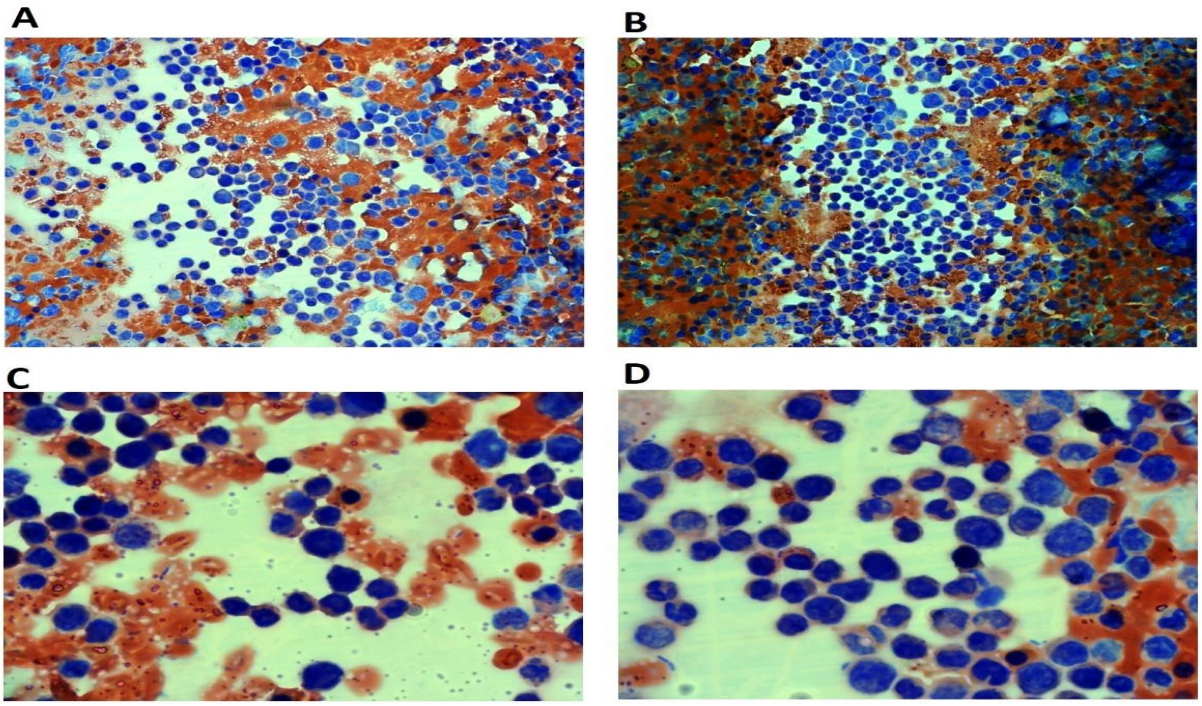

Figure 1A – 1D.
Analysis of the blood hematology in primate model.

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Histopathological evaluation of bone marrow smears stained using the May-Grünwald-Giemsa method revealed a consistent and physiologically normal structure across all samples. However, there was a noticeable increase in cell density within the experimental group (Fig. 1A–D). To further investigate the potential effects of Synthesit iron citrate on blood cell counts in primates, we conducted a study using rhesus macaques aged between 22 and 25 years. These older primates were selected to assess the supplementation's ability to promote hematopoiesis and maintain blood cell and biochemical profiles within physiological ranges in mature animals. The study involved administering Synthesit iron citrate orally, diluted in water, to the rhesus macaques. Blood samples were collected at various time points: on day 0 (baseline), on days 15, 29, and 43 of supplementation, and one week after supplementation was discontinued (day 50). This comprehensive sampling schedule allowed for a thorough analysis of the supplementation's effects over time.

Figure 2A to 2F illustrates the hematological changes observed during Synthesit iron citrate supplementation. Initially, there was a significant decrease in erythrocyte count until day 29, followed by a gradual increase almost returning to baseline levels by day 50 (Fig. 2A). Hematocrit levels showed a non-significant decrease in the first half of the study but significantly rose in the second half, reaching a peak by day 53 (Fig. 2B). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and hemoglobin concentration did not show significant changes at the end of the supplementation period, although there was a transient decrease in hemoglobin levels at day 32, which subsequently returned almost to baseline levels by the end of the study (Fig. 2C, 2D). Thrombocyte count exhibited a significant increase during the first two weeks of Synthesit iron citrate intake, followed by a slight decrease after two weeks but still remaining higher than baseline levels. However, after the termination of supplementation, there was a significant decrease in thrombocyte count between day 15 and day 50 (Figure 2F).

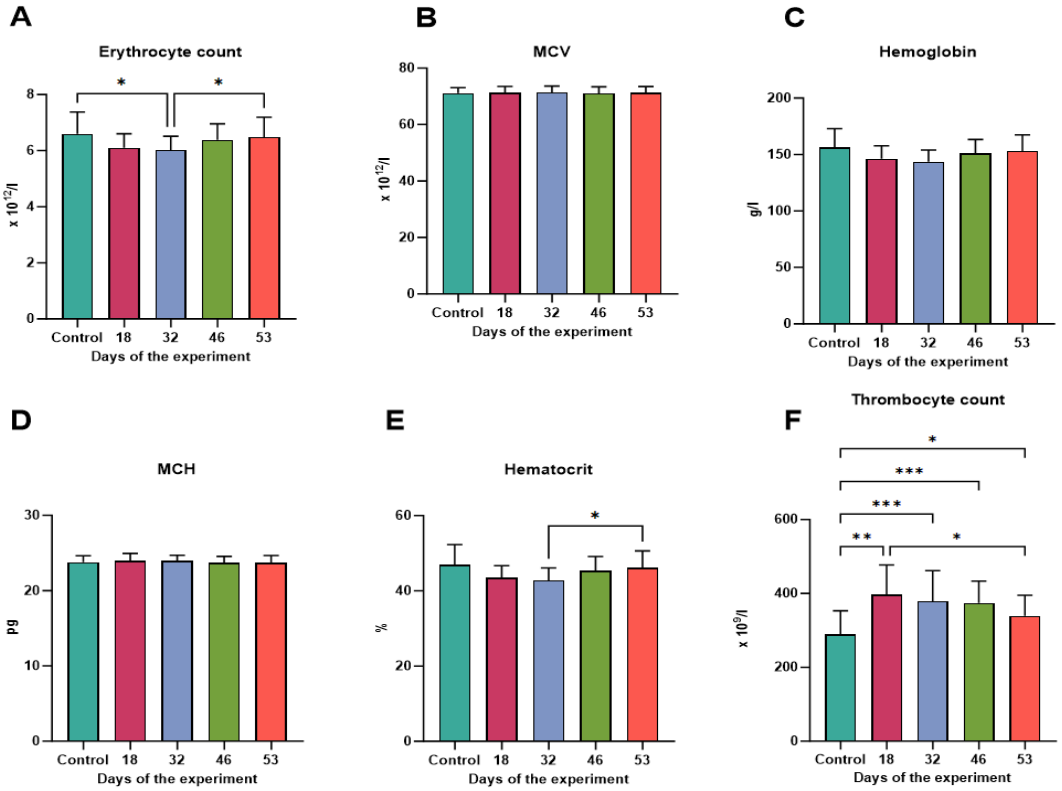

Figure 2A – 2F.
Hematological changes during Synthesit iron citrate supplementation.

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During a study involving rhesus macaques, the effects of Synthesit iron citrate supplementation on peripheral blood leukocyte cell percentages were examined. Initially, there was a slight decrease in the total leukocyte count over two weeks of supplementation, followed by a significant increase between the 15th and 50th day. Specifically, the count rose from 8.94 ± 2.09 x 10^9/l to 10.80 ± 2.89 x 10^9/l during this period (adjusted p = 0.0098, 95% CI 1.947 to 10.75) (Fig. 3A). Regarding specific leukocyte cell percentages, a reduction in monocyte percentage was observed on day 50 compared to both baseline and the two-week mark. The baseline percentage was 5.27 ± 1.49%, which decreased to 4.68 ± 0.90% on day 14 and further to 3.775 ± 1.13% on day 50 (adjusted p baseline – day 50 = 0.0404, day 15 – day 50 = 0.0386, 95% CI baseline – day 50: 0.06879 to 2.931, 95% CI Day 15 – day 50: 0.05005 to 1.750) (Fig. 3B). The lymphocyte percentage significantly decreased after four weeks of supplementation and remained low after the supplementation period ended, before showing a slight increase. Specifically, it decreased from 45.85 ± 8.00% to 35.51 ± 6.68% on day 32 and 34.88 ± 7.371 on day 46 (Fig. 3C). Neutrophil percentage increased steadily throughout the supplementation period, with significant increases observed between baseline and days 29, 43, and 50. The baseline percentage of 45.54 ± 7.99% rose to 57.18 ± 7.83% on day 29 (adjusted p <0.0001, CI -14.59 to -8.689), 57.13 ± 9.27% on day 43 (adjusted p = 0.0014, CI -17.54 to -5.636), and 54.26 ± 7.49% on day 50 (adjusted p = 0.0008, CI -12.84 to -4.615) (Fig. 3D). The percentages of eosinophils and basophils remained consistent with baseline parameters throughout the study period, showing no significant increase or decrease (Fig. 3E,3F).

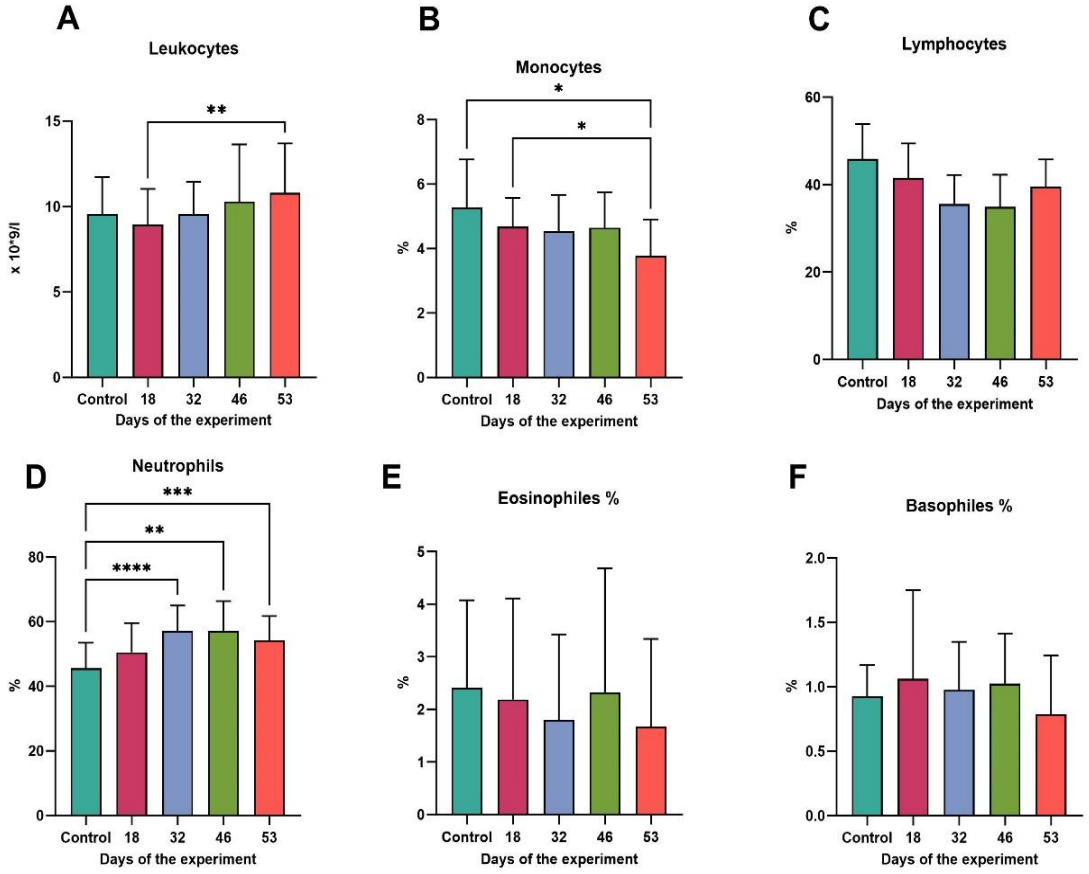

Figure 3A – 3F.
Changes in peripheral blood leukocyte cell percentage in rhesus macaques following synthesit iron citrate supplementation.

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The study investigated the effects of Synthesit iron citrate supplementation on blood biochemical profiles in primates, examining parameters such as glucose, metabolites (cholesterol, triglycerides, creatinine, bilirubin), ions (sodium, potassium), enzymes (alkaline phosphatase, AST, ALT), and proteins (total protein, albumin, globulin). Additionally, levels of free iron were assessed to understand absorption patterns. Analysis revealed differential alterations in these parameters at various time points: early (day 15), middle (day 28), and late (day 42) during supplementation. Effects on certain parameters, including glucose concentration, thrombocyte count, and cholesterol concentration, persisted even after supplementation cessation (day 50). Notably, a significant decrease in glucose levels was observed by the end of supplementation (baseline: 4.36 ± 0.32 mmol/l vs day 43: 3.30 ± 1.06 mmol/l, adjusted p = 0.0491, CI 0.004245 to 2.121). Triglyceride levels also decreased significantly after four weeks of supplementation (baseline: 1.49 ± 0.63 mmol/l vs day 29: 0.70 ± 0.34 mmol/l, adjusted p = 0.0170, CI 0.1598 to 1.425). However, cholesterol levels exhibited a different trend, initially decreasing significantly after four weeks (baseline: 4.563 ± 0.799 mmol/l vs day 29: 2.84 ± 0.88 mmol/l, adjusted p = 0.0043, CI 0.6485 to 2.784) but subsequently rising by the end of supplementation (day 42), though remaining lower than baseline. While bilirubin and creatinine levels showed a slight decline after 29 days of supplementation, the overall decrease was not significant.

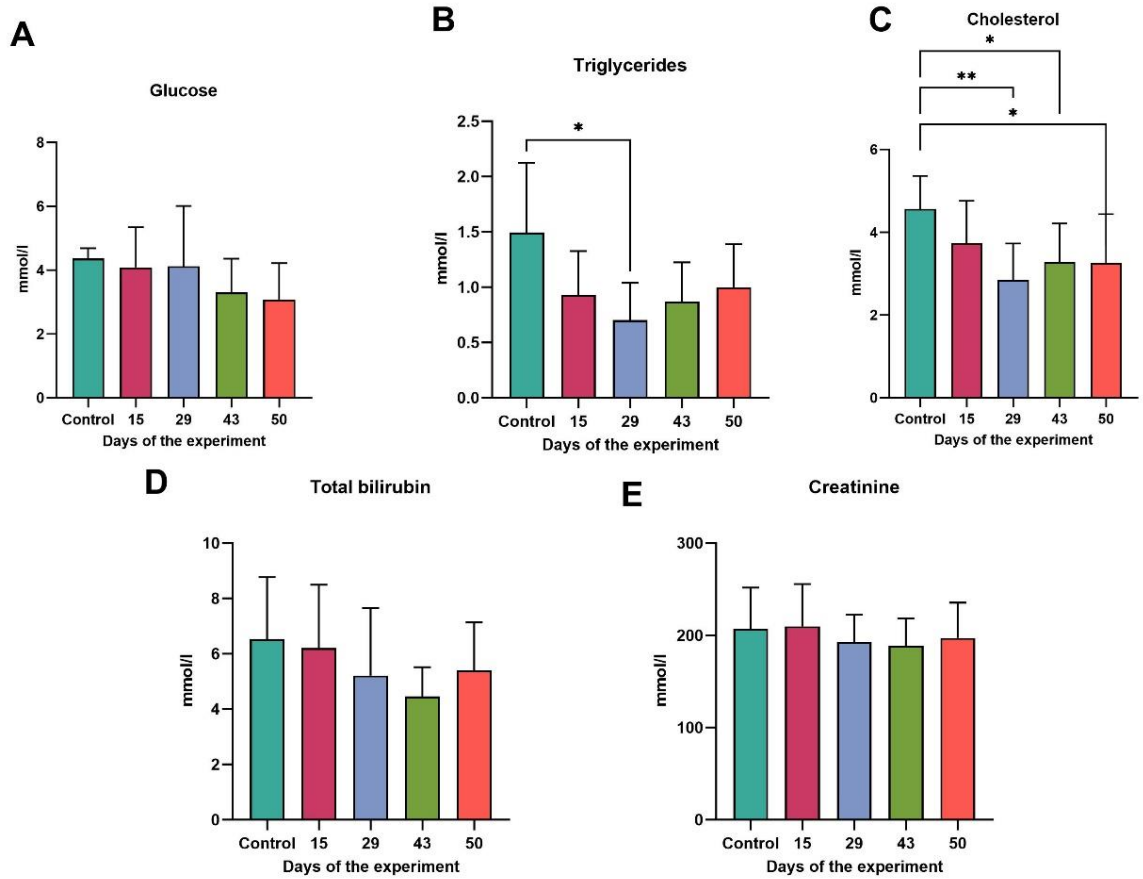

Figure 4A – 4E.
Blood biochemistry in primates.

During the course of nutritional supplementation, the observed dynamics indicated a reduction in levels of potassium and free iron from day 15 onwards, with the effect tapering off by the end of the supplementation period (Fig. 5A, 5B). Specifically, there was a significant decrease in free iron levels

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until day 29 compared to baseline levels (baseline: 22.76 ± 3.61 mmol/l vs day 29: 17.41 ± 4.23 mmol/l, adjusted p = 0.0150, 95% CI 1.181 to 9.519), and this concentration remained relatively stable until the end of supplementation (Fig. 5B). Notably, no significant changes were observed in sodium levels. Although there was a trend of decreasing total protein and globulin levels until day 29, with subsequent growth noted at the end of supplementation (day 43), these changes did not reach statistical significance (Figure 5C–E). However, albumin levels did show a significant decrease after four weeks of supplementation (41.13 ± 2.30 g/l vs 34.25 ± 5.18 g/l, adjusted p = 0.0135, CI = 1.618 to 12.13) (Figure 5F).

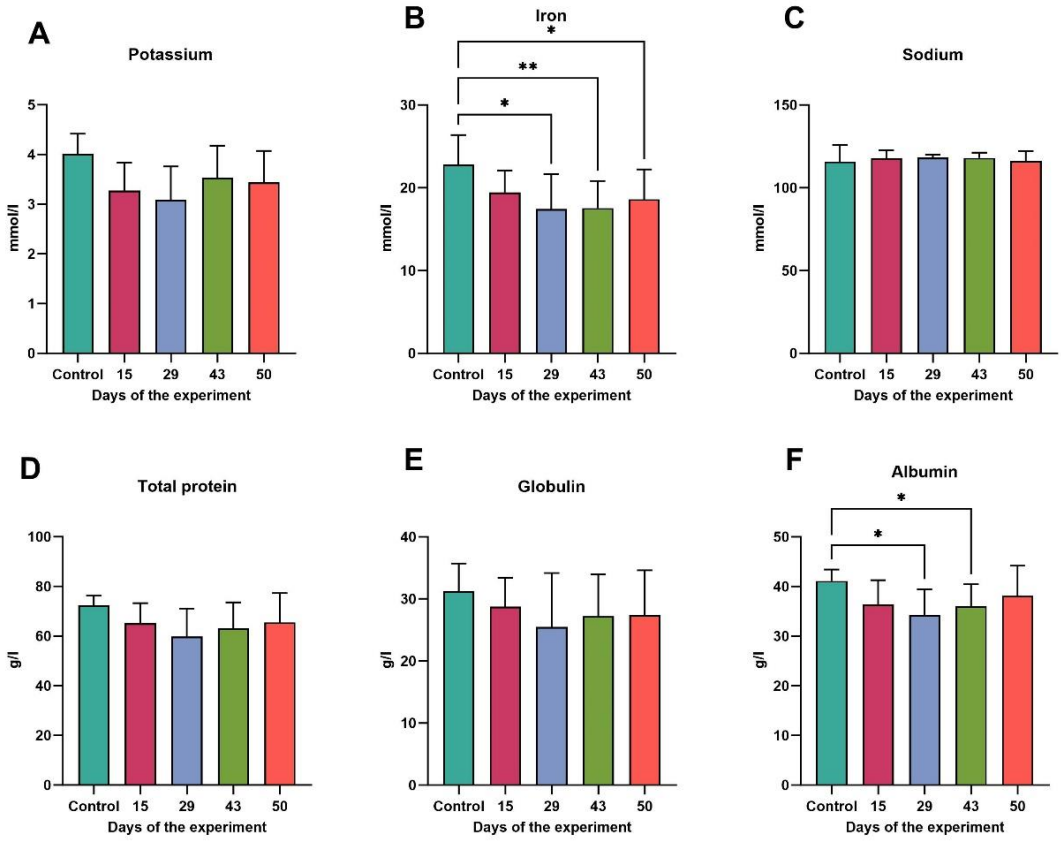

Figure 5A – 5F.
Dynamics of Serum Electrolytes and Protein Levels During Nutritional Supplementation.

In this study, various parameters associated with metabolic disorders were examined, as they often correlate with cardiovascular issues, metabolic syndrome, and diabetes, and are commonly utilized in clinical diagnostics. The focus was on assessing the effects of Synthesit iron citrate on these biomarkers in macaques over a span of 50 days. The results indicated positive improvements in markers of liver damage, glucose, and cholesterol, suggesting overall physiological enhancements. Throughout the experiment, which spanned 30 days for mice and 50 days for macaques, the safety profile of Synthesit iron citrate was evaluated. No alterations in animal behavior were observed, and macaques' water intake remained stable. Notably, a significant decrease in urea concentration in blood was observed on days 15 and 29 of supplementation but not in subsequent time points. Furthermore, analysis of liver and muscle enzymes, including ALT, AST, and ALP, showed significant reductions after supplementation. These enzymes are indicative of liver damage and their lowered levels suggest potential benefits in mitigating conditions like non-alcoholic fatty liver disease (NAFLD). Additionally, observations on weight and

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food consumption in macaques indicated a slight reduction in food intake and a significant decrease in body weight, suggesting potential metabolic effects of the supplementation. Overall, these findings provide valuable insights into the potential physiological improvements and safety of Synthesit iron citrate supplementation in macaques.

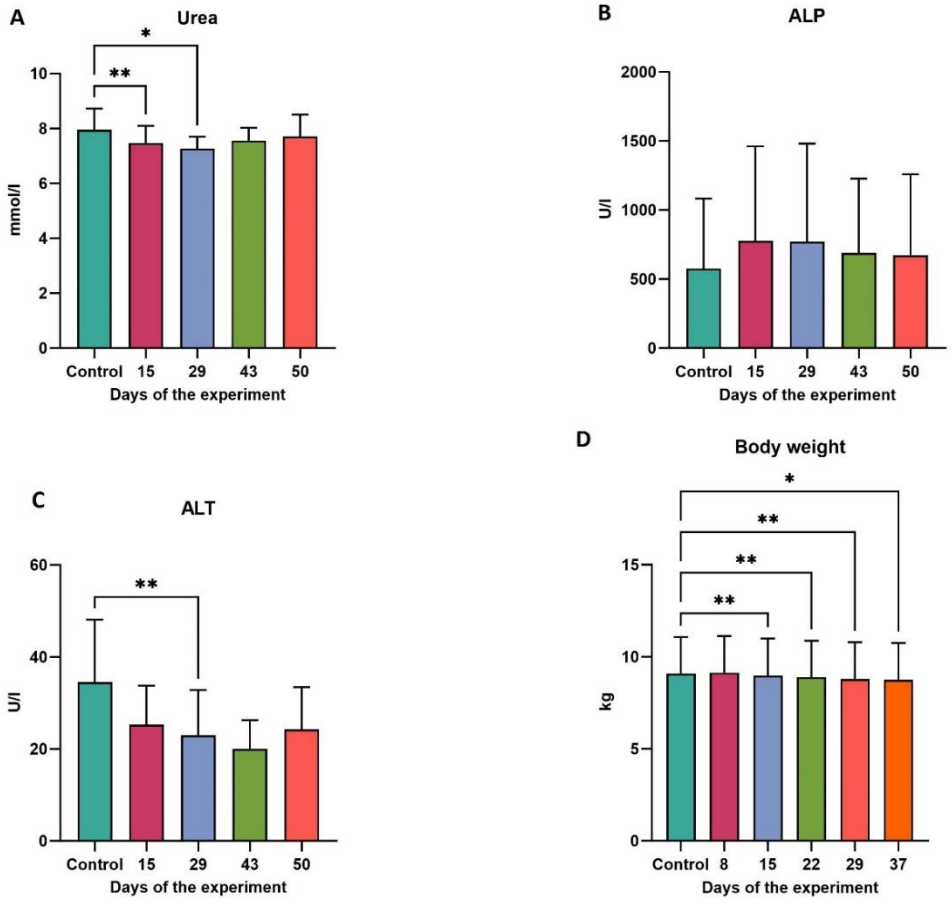

Figure 6A – 6D.
Impact of supplementation on primate weight: Histopathological observations, toxicity studies, and immunogenic potential.

The biosafety of Synthesit iron citrate was assessed using a combination of in vitro and in vivo models. In murine models, hearts, lungs, and spleens were examined for any changes in size or tissue structure compared to the control group, with no observed alterations noted (Fig. 7A–7C). Additionally, primary human skin fibroblasts were subjected to MTT analysis to evaluate cytotoxic effects over a 72-hour period, revealing no acute toxicity (Fig. 7D). Gene expression analysis of proinflammatory cytokines IL6, IL1beta, and CCL2 showed a reduction in mRNA levels upon exposure to Synthesit iron citrate (Fig. 7F). Microscopic examination of tissues from mice control and experimental groups, including myocardia, lung, and spleen, displayed no discernible differences in structure or morphology (Fig. 7E–7G). These findings collectively indicate the biosafety of Synthesit iron citrate, as evidenced by the absence of pathological alterations across various models, suggesting its potential suitability for use as an iron-containing supplement.

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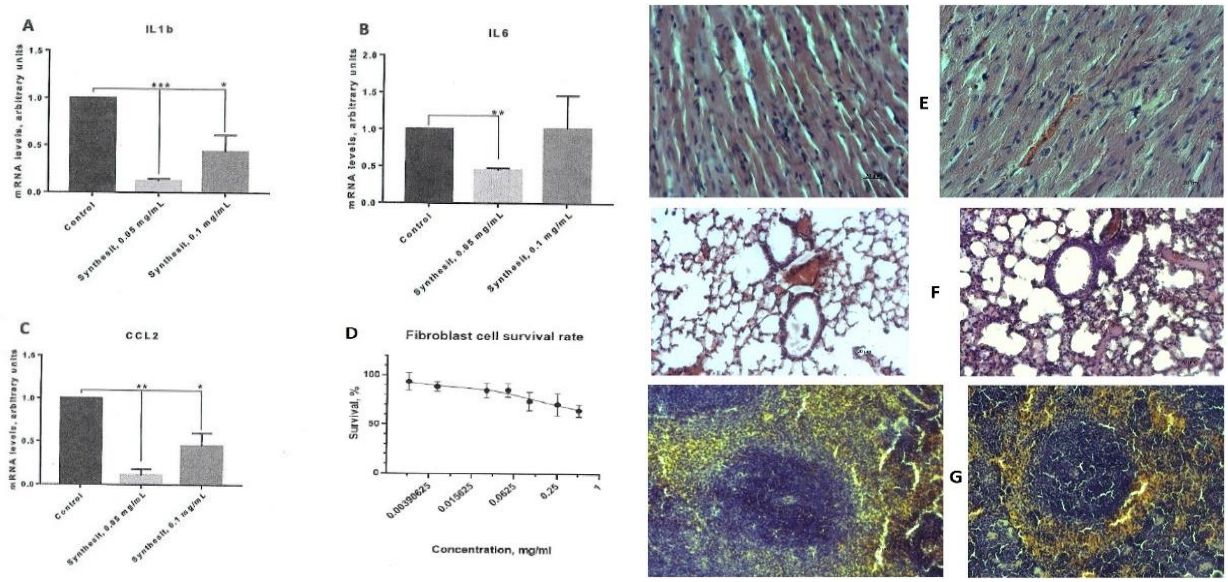

Figure 7A – 7G.
Biosafety evaluation of the Synthesit iron citrate in murine, primate and cell models.

4. Discussion

The current research, carried out by the Research Institute of Medical Primatology in Sochi, Russia, and the Scientific Research Center Synthes-tech, examined the impact of Synthesit® iron citrate supplementation on physiological parameters in elderly rhesus macaques. These monkeys, which age at a rate almost three times quicker than humans and exhibit similar indicators of aging, were chosen as optimal subjects for aging study. The study aimed to specifically examine the effects of Synthesit® iron citrate on age-related problems in monkeys aged 22-25 years who were not iron deficient. This research is consistent with other studies that seek to identify interventions for age-related conditions. Its goal is to deepen our understanding of the aging process and provide potential ways to improve the health and overall quality of life in aging populations. Additionally, it examines the effects of iron citrate on non-iron deficient conditions [27,28].

The recent study on the impact of taking Synthesit iron citrate orally on the production of blood cells in mice provides important new information about how iron affects the function of blood cell formation. The results indicate that the addition of Synthesit iron citrate considerably increases the rate of cell division and stimulates the growth of several types of blood cells in the bone marrow of mice, namely in the production of platelets and red blood cells, without impacting the number of hematopoietic stem cells. The bone marrow smears stained with May-Grünwald-Giemsa examined histopathologically, showed a normal structure but higher cell density in the experimental group highlighting that orally administering Synthesit iron citrate demonstrated potential in stimulating the production of blood cells and maintaining normal levels of blood cells and biochemical substances. These findings are consistent with prior research that emphasizes the significance of iron in the process of hematopoiesis. Studies have demonstrated that a lack of iron can interfere with the development of blood cells in embryos by hindering the growth and specialization of specific types of blood cell precursors, particularly those involved in the production of red blood cells. This highlights the crucial function of iron in facilitating the initial phases of hematopoietic development [29]. Additionally, the complex connection between the balance of iron inside cells and the functioning of blood cell production is emphasized by studies on how FBXL5 controls iron levels. FBXL5 is essential for the maintenance of hematopoietic stem cells and for the prevention of cellular harm caused by oxidative stress. Hematopoietic failure in disorders like myelodysplastic syndromes has been linked to the

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downregulation of FBXL5 in HSCs [30]. When looking at all of these studies together, it becomes clear that iron has multiple roles in controlling hematopoiesis. The relevance of its role in maintaining a healthy hematopoietic system is emphasized by its ability to regulate the proliferation, survival, and differentiation of many hematopoietic cell types. The latest study on Synthesit iron citrate supplementation contributes to the increasing amount of evidence that supports the therapeutic potential of adjusting iron levels in disorders related to disturbed hematopoiesis. To summarize, the study highlights the significance of comprehending the intricate relationship between iron levels, cellular balance, and blood cell production. By clarifying the mechanisms by which iron affects hematopoiesis, scientists can pinpoint possible treatment targets for illnesses defined by imbalanced iron levels and failure of blood cell production. This not only improves our comprehension of hematopoietic control but also creates opportunities for the creation of new therapeutic therapies.

The current study reveals compelling findings concerning its impact on several blood parameters. The initial decline in red blood cell counts and hematocrit levels, followed by a steady improvement, together with variations in platelet count, demonstrate the intricate relationship between iron supplementation and blood-related measurements. Examining these findings in relation to previous research offers significant contextual information. Similarly, another study on ferric citrate supplementation have shown a drop in red blood cell count, which is consistent with the initial decline reported in erythrocyte count in the Synthesit trial [31]. Other study also suggests a potential mechanistic link between the two forms of iron citrate and their impact on erythropoiesis. Furthermore, the interaction between iron supplementation and vitamin B6, as indicated in long-term supplementation studies, emphasizes the importance of considering synergistic effects when assessing hematocrit levels and other blood parameters [32]. Such interactions could contribute to the observed variability in hematocrit levels during the study period with Synthesit supplementation. Additionally, insights from research on oral iron absorption mechanisms, particularly involving ferric citrate hydrate and hepcidin-25, shed light on how different forms of iron are absorbed and their consequent effects on blood parameters [33]. Synthesit® iron citrate boosts hematopoiesis in murine models, vital for post-viral or therapeutic immune recovery. Increased megakaryocytes and thrombocytes suggest a significant role in blood cell regulation [34] . So understanding these mechanisms is crucial for elucidating the fluctuations in erythrocytes, hematocrit, and thrombocytes observed with Synthesit supplementation. Variability in absorption rates and bioavailability among different iron formulations likely contributes to the diverse hematological responses seen in the study. Integrating findings from these related studies offers a more comprehensive understanding of the impact of iron citrate supplementation on blood parameters. This broader perspective not only enhances our knowledge of the underlying mechanisms but also provides insights for refining supplementation strategies to optimize patient outcomes in clinical settings. By considering the various factors influencing hematological responses to iron citrate supplementation, clinicians can tailor interventions more effectively to individual patient needs, thereby improving the management of iron deficiency and related conditions.

The current research further reveals fascinating observations on the intricate relationship between iron metabolism and the dynamics of immunological response in primates. The fluctuations in the overall count of leukocytes and changes in the percentages of different types of leukocytes after taking iron citrate supplements indicate a complex connection between the availability of iron and the modification of the immune system. The initial fall in the total leukocyte count, followed by a considerable increase between days 15 and 50 after supplementation, highlights the dynamic character of leukocyte dynamics driven by the consumption of iron citrate. This fluctuation may indicate an adaptive reaction of the immune system to variations in iron availability, possibly indicating alterations in the production of blood cells or the movement of immune cells. Furthermore, the precise alterations in the proportions of different types of white blood cells offer additional understanding of the possible mechanisms that explain the observed impacts. The gradual decline in the proportion of monocytes indicates a possible adjustment in the production or replacement of monocytes, which could be driven by changes in iron metabolism. The varied patterns shown in the percentages of lymphocytes, neutrophils,

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eosinophils, and basophils demonstrate the specific effects of iron citrate supplementation on different types of immune cells. Another study investigating iron metabolism and the effects of supplementing in primates examines the influence of ferric ammonium citrate on the survival of cells and emphasizes the potential ramifications of changes in iron levels on the functioning of immune cells [35]. Moreover, studies on the transfer of iron from mother to fetus provide insights into the overall impact of iron supplementation on the body, emphasizing the importance of comprehending how iron moves through biological barriers and affects immune responses [36]. Furthermore, research on the systemic impacts of various types of iron supplementation, such as iron dextran, highlights the wider metabolic consequences of iron supplementation on immune function. The changes in the serum metabolomic profile reported in young rhesus monkeys after iron dextran feeding demonstrate the relationship between iron levels and metabolic pathways, which can indirectly affect immunological responses [37]. A further study found that the buildup of iron generated by Hepcidin increases the production of CXCL1 in keratinocytes, which in turn leads to the recruitment of neutrophils in necrotizing fasciitis [38]. One study demonstrates that Deferasirox, an iron chelator licensed by the FDA, efficiently lowers inflammation caused by neutrophils by depleting iron reserves. Supplementing primates with Synthesit® iron citrate increases the overall number of white blood cells, with a preference for producing neutrophils while reducing the number of lymphocytes and monocytes. This aligns with iron's influence on the creation of blood cells [39]. Furthermore, Iron not only hinders the differentiation and activity of Th1, Th2, and Th17 cells, but also serves as an adjuvant for Th1 and Th2 immune responses. The complex interaction between iron and the immune system emphasizes iron's ability to both modulate and potentially be used as a therapy [40]. Overall, the results of the study on iron citrate supplementation in rhesus macaques are consistent with previous research on iron metabolism and the impact of supplementation in primates. The complex interplay between iron levels, populations of immune cells, and the overall immunological response highlights the significance of taking into account the status of micronutrients when studying immune function in non-human primates. Additional research is necessary to understand the molecular processes that explain these data and completely grasp the effect of iron supplementation on the immunological dynamics of primates.

The study on the supplementation of synthetic iron citrate in primates provides interesting insights into its possible impact on metabolic parameters. The observed enhancements in glucose and triglyceride levels, along with a transient decrease in cholesterol, indicate a potential approach to addressing metabolic health. The enduring effects observed after supplementation for glucose and triglycerides suggest long-lasting advantages, while there is a minor increase in cholesterol levels. These findings are consistent with another study conducted on HepG2 cells and primary hepatocytes, which showed that reactive oxygen species (ROS) cause lipid buildup, leading to increased production of cholesterol and triglycerides [41]. Another study corroborated the findings of improved glucose and triglyceride levels after taking Synthesit iron citrate supplements. This study also emphasized that a diet low in iron reduces serum triglycerides and cholesterol levels, further supporting the idea that iron levels affect lipid profiles[42]. Furthermore, studies suggest that excessive iron levels can disturb the balance of glucose in the body, contribute to the accumulation of fat in the liver, and increase the levels of triacylglycerols in the blood of rats [42]. These findings emphasize the crucial role of iron levels in metabolic health, which may be relevant to the effects of Synthesit iron citrate supplementation observed in primates. The intricate interplay between iron levels, lipid metabolism, and glucose regulation underscores the complexity of these metabolic pathways. While the initial metabolic benefits of Synthesit iron citrate supplementation are promising, further investigations are warranted to comprehensively evaluate its long-term effects and the underlying mechanisms driving the observed changes in metabolic parameters. This highlights the need for continued research to elucidate the full spectrum of effects and potential implications for metabolic health.

The current study found a notable decrease in potassium and free iron levels in the serum electrolyte and proteins. This reduction began on day 15 and stabilized at the conclusion of the term. Significant reductions were observed in albumin levels, whereas sodium levels remained unaltered.

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Although there were initial declines in overall protein and globulin levels until day 29, later growth was recorded, suggesting an intricate interaction between supplementation and biochemical indicators. Furthermore, another study emphasized the usefulness of dietary iron supplementation in resolving this disease, underscoring its value [43]. The importance of implementing effective iron fortification strategies was highlighted as a progressive and sustainable means of enhancing iron levels in populations experiencing dietary deficits. The observed alterations in potassium, liberated iron, and other biochemical indicators resulting from nutritional supplementation underscore the complex correlation between nutrients and the body's physiological activities. Overall, the study's findings offer valuable understanding of the ever-changing characteristics of biochemical indicators when exposed to nutritional supplementation. By using these discoveries in conjunction with prior investigations, we can enhance our comprehension of the impact of nutrients on physiological mechanisms and devise more efficient approaches to tackle nutritional inadequacies.

The beneficial impacts of generating iron citrate on metabolic indicators in macaques across a 50-day duration are remarkable, specifically in relation to enhanced liver function, glucose, and cholesterol levels. These discoveries are important because they indicate the possibility of improving the physical abilities of monkeys by controlling their iron metabolism. Another related work on the SYNTHESIT of hepcidin, a crucial regulator of iron metabolism, provides a more comprehensive understanding of these consequences. Hepcidin is crucial in regulating iron balance by detecting several signals that indicate iron levels and erythropoietic activity. This regulatory mechanism is likely to affect the metabolic improvements found in macaques after iron citrate production. For example, the transitory declines in urea concentration and large reductions in liver and muscle enzymes may be associated with hepcidin-mediated alterations in iron metabolism. These modifications may enhance liver function and metabolic factors, such as glucose and cholesterol levels [44]. Additionally, findings on the correlation between weight and food consumption in macaques indicate possible metabolic advantages linked to the production of iron citrate. This is consistent with the general understanding of how iron metabolism affects metabolic processes and overall physiological health. Another study has demonstrated the complex connection between iron, metabolic pathways, and health outcomes, emphasizing the necessity for additional research to reveal the underlying mechanisms of these effects [45]. Overall, the beneficial impacts of iron citrate production on metabolic indicators in macaques highlight the complex relationship between iron metabolism and physiological well-being. By examining relevant research on the SYNTHESIT of hepcidin, we may get a more comprehensive comprehension of the mechanisms that drive these effects. This expanded viewpoint emphasizes the possibility of doing additional research and clinical studies to utilize the manipulation of iron metabolism for enhanced health outcomes in primates and maybe in humans as well.

The present study also performed a comprehensive safety assessment of Synthesit iron citrate, including extensive examinations conducted both in laboratory settings (in vitro) and in living organisms (in vivo), which yielded encouraging findings. There were no discernible alterations in the structure of the heart, lungs, or spleen in murine models as compared to the control groups. Similarly, when human skin fibroblasts were exposed to Synthesit iron citrate, there was no immediate harmful effect observed. Gene expression analysis revealed decreased levels of proinflammatory cytokines, indicating a potential anti-inflammatory impact. Microscopic investigation confirmed these findings by showing that there were no discernible structural variations in the tissues. Furthermore, another study conducted a detailed evaluation of the safety characteristics of Synthesit iron citrate and obtained comparable findings. Moreover, it is important to take into account further research findings about iron citrate complexes, specifically a study on their nuclearity at levels that are biologically significant [46]. Comprehending the chemical composition of these complexes is crucial for forecasting their interactions and potential toxicity. Furthermore, studies on the therapeutic properties of iron citrate, specifically its ability to inhibit calcium deposition, may provide useful insights into its possible health advantages and interactions with other minerals in the body [47]. To summarize, the evaluation of Synthesit iron citrate showed encouraging results regarding its safety. However, to gain a more complete

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understanding of its safety profile and potential benefits as a food supplement or additive, it is advisable to consider data from studies on the nuclearity of iron citrate complexes, its therapeutic effects, and the FDA's safety determinations. An integrated strategy is crucial for making well-informed decisions on the utilization of Synthesit iron citrate in different applications.

Finally, the addition of Synthesit® Iron citrate has a notable impact on hematopoiesis, specifically via increasing the formation of thrombocytes in both murine and primate models. In addition to affecting blood-related changes, it also has an impact on several biochemical markers such as triglycerides, glucose, hepatic enzymes, and levels of free iron. This indicates potential consequences for cardiovascular and metabolic well-being. These data suggest that it is important to carefully think about the use of iron supplements in clinical practice, paying attention to the amount, length of time, and possible impacts on groups that are not deficient in iron. Thorough review is essential to negotiate the complexity of iron supplementation and minimize any unintended implications in patient care.

5. Conclusion

In conclusion, the comprehensive exploration of Synthesit® iron citrate supplementation across current study offers a promising insight into its multifaceted effects on physiological parameters in both murine and primate models. Through meticulous investigation, it becomes evident that Synthesit® iron citrate plays a significant role in modulating hematopoiesis, particularly by enhancing thrombopoiesis and erythropoiesis without affecting hematopoietic stem cell count. Moreover, its impact on metabolic parameters such as glucose, triglycerides, and cholesterol underscore its potential in addressing metabolic health concerns. The observed fluctuations in biochemical markers and immune cell populations following supplementation highlight the complex interplay between iron metabolism and immune response dynamics, suggesting a nuanced relationship that warrants further exploration. Additionally, the biosafety assessment demonstrates promising results, providing a foundation for considering its potential applications as a food supplement or additive. However, to fully harness the benefits of Synthesit® iron citrate supplementation, careful consideration of dosage, duration, and potential effects in non-iron deficient populations is essential. Integrating findings from related studies enhances our understanding of its safety profile and potential therapeutic benefits, emphasizing the importance of informed decision-making in clinical practice. In summary, Synthesit® iron citrate supplementation holds significant promise in modulating hematopoiesis, metabolic parameters, and immune response dynamics, with implications for cardiovascular, metabolic, and overall physiological health. Further research is warranted to elucidate its mechanisms of action, optimize supplementation strategies, and ensure its safe and effective utilization in various clinical settings.

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Authors' contributions:

The study was conceived and designed by Patrik Kusnir and Dmitry Bulgin. They also carried out the Synthesit of iron citrate and performed all laboratory experiments. Shahbaz Baig contributed to analyzing and interpreting the results on how iron citrate affects blood parameters in non-iron deficient animals. Both authors were involved in drafting the initial manuscript, critically revising it, and reviewing the final version for approval prior to publication.

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