



## Article

# The Impact of Krebs Cycle Intermediates on the Endocrine System and Immune System: A Comparison

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**Abstract:** Introduction: The Krebs cycle is an important set of reactions that synthesize different molecules and substances that affect various organs. The objective of this paper was to compare the effects of Krebs cycle intermediates on the endocrine system and the immune system. Methods and Materials: The articles used in this paper were obtained from a systematic search of PsycINFO, PubMed, Web of Science, CINAHL, and primary databases. The search terms were “Krebs cycle,” “intermediates,” “endocrine system,” “tricarboxylic acid,” “citric acid cycle,” and “immune system,” and Boolean operators (AND/OR) were used to combine terms. Results: A review of the selected studies showed that Krebs cycle intermediates influence how the endocrine system regulates and controls body processes, including energy uptake. Moreover, these intermediates have both direct and indirect effects on immune function, memory, and activation. Discussion: An understanding of the effects of Krebs cycle intermediates on endocrine and immune processes will provide valuable insights for the development of new therapies. Additionally, this knowledge is a basis for exploring the pathogenesis of the complications related to endocrine system function and for evaluating the immune system response to pathogens. Conclusions: The evidence gathered in this review shows that Krebs cycle intermediates have significant effects on immune and endocrine processes. However, further human and in vivo studies are required to generate additional evidence for the underlying pathways and to identify the potential strategies for targeting these mechanisms to manage specific disorders.



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**Keywords:** Krebs cycle; biochemistry; regulation; hormones; metabolites; endocrinology; immune system

## 1. Introduction

In the last decade, there has been a significant increase in the number of studies on intracellular metabolic processes and on how immune cell behavior depends on energy demands and the presence of a disease-causing pathogen [1,2]. Moreover, there have been attempts to examine the specific effector mechanisms responsible for different cell behaviors [2]. One area of recent research focus is the Krebs cycle, also referred to as the tricarboxylic acid (TCA) cycle or the citric acid cycle (CAC) [2,3]. The Krebs cycle is the primary acetyl-CoA oxidative pathway that results in the formation of reducing agents (NADH and FADH<sub>2</sub>) in anaerobic organisms [4,5]. Other researchers have described the Krebs cycle as a metabolic process in the mitochondrial matrix of every aerobic organism [5,6]. Acetyl-CoA is formed by the breakdown of different nutrients, such as glucose, and is then funneled into the TCA pathway.

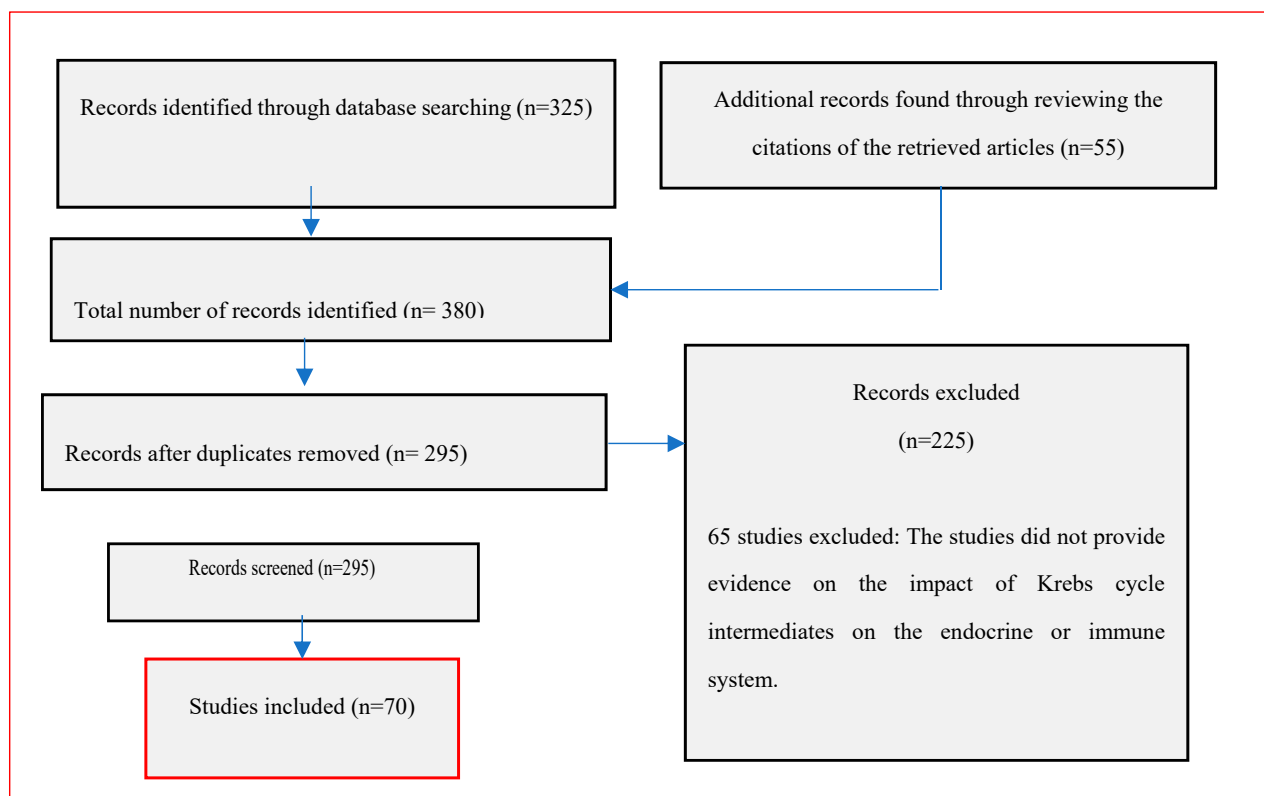
Several enzymatic reactions are required to complete the entire cycle. Most of these reactions are catalyzed by enzymes such as citrate synthase (CS), aconitase (ACO2),  $\alpha$ -ketoglutarate dehydrogenase (OGDH), succinate dehydrogenase (SDH), malate dehydrogenase (MDH), fumarase (FH), isocitrate dehydrogenase (IDH), and succinyl-CoA synthetase [6,7]. The primary function of these enzymes is to catalyze the reactions that result in the secretion of the TCA products NADH and FADH<sub>2</sub> [8]. Both NADH and

FADH<sub>2</sub> can transfer electrons to the electron transport chain (ETC) to help drive oxidative phosphorylation (OXPHOS) [3,9,10]. In addition, the transferred electrons are involved in the synthesis of the nucleoside triphosphate adenosine triphosphate (ATP) [11–13].

Importantly, electron transfer to the ETC usually occurs through redox reactions that facilitate the creation of an electrochemical proton (H<sup>+</sup>) gradient and the subsequent synthesis of ATP by ATP synthase [14–16]. The conversion of lipid and protein molecules into ATP is an oxidative (OXPHOS) process and leads to the formation of carbon dioxide as a byproduct. The objective of this paper was to compare the impacts of Krebs cycle intermediates on the endocrine system and the immune system.

## 2. Methodology

The current study examined the relationship between the Krebs cycle and the endocrine and immune systems. The review process included an analysis of the search results from PsycINFO, PubMed, Web of Science, and CINAHL. The search terms and phrases were “Krebs cycle,” “intermediates,” “endocrine system,” “tricarboxylic acid,” “citric acid cycle,” and “immune system,” and Boolean operators (AND/OR) were used to combine search terms to identify additional sources of evidence for this systematic review. The search was limited to articles that were published in the four electronic databases between 2015 and 2019. The abstracts of the available articles were carefully reviewed to determine their quality and appropriateness (see Figure 1). The initial search yielded approximately 1957 articles. Other parameters, including the study type, publication year, text options, search field tags, and language, were used to limit the search; when these parameters were applied, 325 articles were obtained from the databases, and 55 were obtained through the cross-referencing method. The articles were carefully examined at different stages, as shown in Figure 1, to determine their applicability to this review.



**Figure 1.** PRISMA flow diagram.

### 3. Results

At the end of the search and review process, the final list of articles included animal model studies, clinical trials, experimental studies, and clinical reviews. A total of 70 studies met the inclusion criteria and were considered for review. A summary of the 20 most important references related to the research topic is provided in Table 1. The articles provided vital insights into the relationship between Krebs cycle intermediates and the endocrine and immune systems. An analysis of the studies revealed that the Krebs cycle involves a series of enzymatic reactions that usually catalyze the aerobic metabolism of energy sources such as glucose to water and carbon dioxide, thus synthesizing ATP in the process [2,3,7,8].

The compounds involved in the Krebs cycle act as energy donors or precursors that facilitate the synthesis of lipids, carbohydrates, and amino acids. Krebs cycle intermediates function as energy substrates within the mitochondrial matrix [14] and can exert antioxidative effects on a wide range of systems and organs, including the brain and thyroid gland [14,15]. Therefore, the analysis and understanding of how the Krebs cycle affects the endocrine and immune systems could provide novel therapeutic strategies for disease management.

### 4. Impact of Krebs Cycle Intermediates on the Endocrine System

The endocrine system is an important chemical messenger network composed of hormonal feedback loops between internal organs and the circulatory system that help regulate specific organs [11]. In humans, the endocrine system encompasses the thyroid gland and the adrenal gland and depends heavily on mitochondrial function as the primary source of energy for cell survival and proliferation [11].

The mitochondrial network is highly flexible, thus enabling cells to adjust to diverse intra- and extracellular conditions such as stress, hypoxia, and nutrient deprivation. The Krebs cycle is an essential part of the mitochondrial network because it unifies the processes of lipid, protein, and carbohydrate metabolism [11,16,17]. Furthermore, the Krebs cycle is the link between most metabolic pathways in cells and mitochondria. The gradient of Krebs cycle products, including NADH and FADH<sub>2</sub>, facilitates the transport of mitochondrial electrons and the synthesis of ATP [11]. In this regard, the Krebs cycle accumulates fuel for the conversion of energy and supports the anabolic processes in the cellular environment [18]. According to Jochmanova and Pacak, dysfunction in the Krebs cycle and its enzymes, including the depletion of associated substrates, can result in disorders and the activation of adaptive processes to support cell survival [11]. Thus, it is evident that the Krebs cycle can affect the regulation of target organs by the endocrine system [11].

In the Krebs cycle, a wide range of intermediates are regenerated at different stages; these intermediates include acetyl-CoA, pantothenic acid, citrate, alpha-ketoglutaric acid, isocitrate, alpha-ketoglutarate, succinate, malic acid, fumarate, pyruvic acid, malate, fumaric acid, and oxaloacetate [11,15,16]. In addition, research has shown how the addition or removal of intermediates affects different endocrine functions, including the regulation of growth and development, metabolism, tissue function, the immune response, and reproduction [17–19]. According to Sinton et al., the addition of Krebs cycle intermediates has an anaplerotic effect, while their removal has a cataplerotic effect [19]. Such changes will increase or decrease the amount of oxaloacetate that combines with acetyl-CoA to form citric acid and thus influence the rate of ATP synthesis. Interestingly, thyroid hormones regulate the ATP synthesis rate in mitochondria [19]. These data imply that an increase in the proportion of Krebs substrates, such as oxaloacetate and acetyl-CoA, leads to an ATP synthesis rate that is higher than needed; at this point, the regulatory function of thyroid hormones may come into play [19,20]. This process highlights a possible link between Krebs cycle intermediates and endocrine function.

According to Lanni et al., the hypermetabolic impact of thyroid hormones as the primary endocrine regulators of metabolic rate is widely recognized [20]; the cellular mechanism that underlies these effects has been extensively examined in recent years. However,

little is known regarding how thyroid hormones regulate diverse cellular functions. A study by Lanni et al. showed that thyroid hormones often have a significant impact on mitochondria in terms of cellular energy synthesis [20]. The regulatory process is hypothesized to involve both genomic and nongenomic mechanisms related to mitochondrial energetics, OXPHOS, and protein uncoupling [21–23]. Furthermore, research has implicated the Krebs cycle products involved in electron transfer, such as NADH and FADH<sub>2</sub>, in the pathways through which thyroid hormones affect mitochondrial function and cellular energy conversion.

Studies on OXPHOS and the ETC have revealed the possible effects of Krebs cycle intermediates on the endocrine system. According to Conley, mitochondria can oxidize substrates to synthesize ATP, which fuels a wide range of body functions, such as locomotion and muscle contraction [22,23]. The ETC sets the oxidation pace and affects the ATP synthesis rate. Research shows that the ETC usually uses the NADH and FADH<sub>2</sub> generated during the Krebs cycle to convert lipids and proteins to ATP [24,25]. OXPHOS is the primary mechanism through which eukaryotic cells synthesize ATP, the primary energy currency of the cell. During the OXPHOS process, electrons are passed through a series of electron transport complexes, particularly respiratory complexes, that are embedded in the inner mitochondrial membrane.

Electron carriers, such as NADH and FADH<sub>2</sub>, donate their electrons to Complex I (NADH dehydrogenase), which passes them down the ETC to Complex II (succinate dehydrogenase), then to Complex III (cytochrome bc<sub>1</sub> complex), and finally to Complex IV (cytochrome c oxidase) [26]. At the end of the ETC, the electrons are transferred to oxygen, the final electron acceptor, forming water as a byproduct. The transfer of electrons through the ETC creates a proton gradient across the inner mitochondrial membrane, which drives the synthesis of ATP. The proton gradient is formed by protons (H<sup>+</sup>), which are pumped out of the mitochondrial matrix into the intermembrane space by Complexes I, III, and IV [27]. This process creates a higher concentration of protons in the intermembrane space compared with the mitochondrial matrix, which forms a proton motive force. This force drives the flow of protons back into the mitochondrial matrix through ATP synthase, a protein complex that spans the inner mitochondrial membrane.

The flow of protons through ATP synthase powers the synthesis of ATP via a process called chemiosmosis. As protons pass through ATP synthase, their energy is used to drive the rotation of a rotor-like structure in the complex, which in turn drives the synthesis of ATP from ADP (adenosine diphosphate) and Pi (inorganic phosphate) molecules [28]. Therefore, the passage of electrons through the respiratory complexes in the ETC creates a proton gradient across the inner mitochondrial membrane, which drives the synthesis of ATP by ATP synthase. OXPHOS is essential for the production of ATP, which provides the energy needed for various cellular processes, such as muscle contraction, biosynthesis, and transport.

Recent research shows that the ETC is also involved in the activity of Complexes I–IV and coenzymes such as ubiquinone and cytochrome c [22–24]. These reactions help transfer H<sup>+</sup> ions to the space between the inner and outer mitochondrial membranes, a process that facilitates OXPHOS. In the presence of oxygen, energy will be passed through electron carriers to synthesize ATP [22–24]. The success of OXPHOS, as well as ATP synthesis during the Krebs cycle, is affected by the presence of thyroid hormones [22,29]. The thyroid hormone has a significant influence on mitochondrial ATP synthesis [30,31]. Similarly, recent research has identified a possible link between metabolic syndrome and thyroid dysfunction. A metabolic disorder can increase the risk of heart complications, obesity, stroke, and type 2 diabetes [32,33].

Patients suffering from metabolic disorders may also experience greater mitochondrial dysfunction, a condition that can significantly reduce the conversion of proteins and lipids into ATP [34,35]. Notably, thyroid dysfunction has been frequently observed in a population of patients with metabolic syndrome [35,36]. A possible explanation is that metabolic dysfunction hinders effective energy and homeostatic regulation in the body. Other researchers have reported that metabolic dysfunction may be caused by changes in

the pyruvate dehydrogenase complex (PDC) [36,37], an important metabolic node that is involved in the oxidation of pyruvate. In addition, the PDC helps drive the Krebs cycle to meet cellular energy demands. In this regard, the altered regulation of pyruvate, a Krebs cycle intermediate, leads to mitochondrial dysfunction and increases the risk of thyroid disorder [37,38]. Further investigations may be required to explore the underlying molecular relationship between these two conditions and the specific Krebs cycle substrates that affect thyroid hormone activity in patients diagnosed with specific disorders.

ATP is the primary energy currency of cells, and ADP is a precursor molecule in ATP synthesis [39,40]. The ATP/ADP ratio is a key determinant of cellular energy status, and it plays a crucial role in regulating ATP synthesis in cells throughout the body, including those making up the tissues and organs of the endocrine system, such as the adrenal and thyroid glands [41]. ATP synthesis occurs through two major pathways, namely, OXPHOS in the mitochondria and substrate-level phosphorylation in the cytoplasm [42]. The former pathway accounts for the majority of ATP production in most cells, including those in the adrenal and thyroid glands. OXPHOS requires a constant supply of electron donors, typically in the form of NADH and FADH<sub>2</sub>, and an oxygen supply to generate a proton gradient across the inner mitochondrial membrane [43]. The proton gradient drives the ATP synthase enzyme, which produces ATP from ADP and Pi. The rate of ATP synthesis is mainly determined by the availability of electron donors and oxygen, which are regulated by a range of factors, including substrate availability, hormonal signals, and metabolic activity.

One of the primary mechanisms through which cells regulate ATP synthesis is through the control of mitochondrial respiration [44]. In cells with high energy demand, such as those in the adrenal and thyroid glands, the rate of mitochondrial respiration is tightly regulated to ensure that ATP production matches energy demands. Therefore, processes such as the regulation of substrate supply, the activity of the ETC, and the proton gradient across the inner mitochondrial membrane can control energy conversion. The ATP/ADP ratio plays a crucial role in this process, as it acts as a feedback signal to modulate mitochondrial respiration. When the ATP/ADP ratio is high, indicating that energy demands are satisfied, cells can reduce mitochondrial respiration and slow ATP production. This situation can be attained through a range of mechanisms, including the inhibition of ETC complexes and the reduction in ATP synthase activity [45]. Conversely, when the ATP/ADP ratio is low, indicating that energy demands are not being met, cells can increase mitochondrial respiration and ATP production to restore energy balance. The upregulation of ETC complexes and the increase in ATP synthase activity facilitate the control of energy.

In addition to its role in regulating mitochondrial respiration, the ATP/ADP ratio also plays a key role in regulating the activity of the enzymes involved in ATP synthesis [46,47]. For example, the rate-limiting enzyme in OXPHOS, cytochrome c oxidase, is known to be sensitive to changes in the ATP/ADP ratio. When the ratio is high, cytochrome c oxidase activity is inhibited, reducing ATP synthesis. Conversely, when the ratio is low, cytochrome c oxidase activity is increased, promoting ATP synthesis. Therefore, it should be noted that the ATP/ADP ratio plays a critical role in regulating ATP synthesis in the cells throughout the body, including those in the endocrine system. This ratio acts as a feedback signal that helps to match energy production with energy demands, ensuring that cells have a constant supply of ATP to fuel metabolic processes. In cells with high energy demands, such as those in the adrenal and thyroid glands, the ATP/ADP ratio is essential in regulating mitochondrial respiration and the activity of the enzymes involved in ATP synthesis.

On the other hand, pancreatic beta-cells play a key role in glucose homeostasis by secreting insulin in response to changes in blood glucose levels [48]. The regulation of insulin secretion is tightly linked to the metabolic state of the beta-cell, and both ATP supply and demand play critical roles in this process. The implication is that the regulation of insulin secretion is a complex process that involves both the supply of ATP to the beta-cell and the demand for ATP within the cell [49]. The reason is that ATP is a key signaling molecule that regulates the activity of ion channels and other proteins involved in insulin secretion. One vital mechanism through which beta-cells regulate ATP supply is through



the oxidation of substrates such as glucose and fatty acids. The oxidation of these substrates in the mitochondria generates electron donors such as NADH and FADH<sub>2</sub>, which drive OXPHOS and ATP synthesis [50]. As noted in the information above, increased substrate supply and oxidation can lead to increased mitochondrial respiration and ATP synthesis, even if the demand for ATP within the cell remains relatively constant.

Notably, the oxidation of glucose and fatty acids leads to a rapid increase in ATP production, even in the absence of glucose-stimulated insulin secretion (GSIS) [51]. The rate of ATP synthesis in the mitochondria is proportional to the rate of electron transport through the ETC [52]. Therefore, the electron transfer complex plays an important role in regulating the pace of oxidation and ATP synthesis. It should be noted that changes in electron transfer complex proteins can affect ATP synthesis and insulin secretion. For example, a genetic defect in the electron transfer complex protein can lead to impaired ATP synthesis and insulin secretion [53]. Additionally, the electron transfer complex protein is elevated in response to glucose, which contributes to the regulation of insulin secretion [54].

It should be noted that metabolic control in pancreatic beta-cells occurs through both ATP supply and ATP demand. Therefore, to some extent, increased substrate supply and consequent oxidation in pancreatic beta-cells can increase mitochondrial OXPHOS independent of significant changes in ATP demand. Additionally, the electron transfer complex sets the oxidation pace, which means that the ATP synthesis rate increases. It is essential to note that ATP supply and demand play significant roles in the regulation of insulin secretion. Additionally, the electron transfer complex is vital in regulating the pace of oxidation and ATP synthesis in beta-cells.

## 5. Impact of Krebs Cycle Intermediates on the Immune System

The link between Krebs cycle intermediates and the immune system has been explored in recent studies. A review of previous research revealed that immune cells traverse different tissues with diverse nutrient and oxygen conditions [38,55,56]. In addition, activated immune cells may change their functional activities in response to the prevailing cellular conditions. For example, lymphocytes can be converted from inert cells to a subpopulation of cells that can synthesize effector molecules such as cytokines [55,57]. Such alterations suggest that metabolic stress must be managed for immune cells to function effectively in different parts of the body. According to Ryan et al., metabolic reprogramming is a critical area of focus for immunologists and biologists because it may be a basis for understanding the pathogenesis of different diseases, such as cancer and diabetes [36]. Moreover, metabolites traditionally linked to biosynthesis and bioenergetics have recently been implicated in immunity and the malignancy of transformed cells [36]. In such studies, the focus has been on how Krebs cycle intermediates, such as succinate, fumarate, 2-hydroxyglutarate isomers (D-2-hydroxyglutarate, itaconate, and L-2-hydroxyglutarate) and acetyl-CoA, affect immune signaling, response, and function [36]. An understanding of the complexities of such metabolic and immune functions may lead to the identification of new therapeutic modalities.

One Krebs cycle intermediate that affects immune system function is succinate. Research has shown that succinate is typically synthesized in the mitochondrial matrix during the Krebs cycle in a process catalyzed by succinyl-CoA synthetase [55–57]. Succinate can accumulate in LPS/IFN- $\gamma$ -treated macrophages and function as a proinflammatory metabolite [55,58]. Inflammatory responses follow different pathways, including the direct secretion of mitochondrial ROS, activation of hypoxia-inducible factor-1  $\alpha$ , and the coupling of G-protein-coupled receptor succinate receptor 1. Littlewood-Evans et al. hypothesized that succinate can function in paracrine and autocrine manners and can increase the production of macrophages [57]. After LPS treatment, intracellular succinate levels and SUCNR1 expression increased.

Another intermediate that affects immune system function is itaconate [57,58], a carboxylic acid with critical anti-inflammatory capabilities. In addition, this metabolite can negatively regulate cytokine production and the inflammatory response in the body [59–61].

According to the study by Lampropoulou et al., an itaconate derivative exhibited a significant capacity to inhibit the proinflammatory function of nitric oxide (NO), ROS, and the cytokines IL-6, IL-12p70, and IL-1 $\beta$  in bone-marrow-derived macrophages [59]. The authors further stated that itaconate could modulate inflammatory responses because it can inhibit succinate and SDH oxidation [59]. Ryan et al. stated that itaconate could function as an inhibitor of SDH activity in macrophages [60]. Furthermore, the inhibition of SDH by itaconate is considered a mechanism for blocking ROS generation by the RET complex [61,62].

Recent studies have further shown that intermediates such as fumarate may also regulate the epigenome, immune cell function, and innate immune memory. Innate immune training is critical for the normal function of monocytes, natural killer cells, and monocytes [63,64]. This process enables these immune cells to develop optimal responsiveness to reinvading pathogens due to increased immunological memory. A study by Arts et al. showed that fumarate accumulation in monocytes was necessary for immune training because it enhanced cytokine production following LPS stimulation [64]. Additionally, the authors concluded that the increased fumarate levels in the immune landscape influence the immune response by inhibiting KDM5 enzymes, thus linking epigenetic regulation and the rewiring of the Krebs cycle. Another study by Blewett et al. showed that dimethyl fumarate (DMF) can inhibit the activation of monomethyl fumarate (MMF) in both human and mouse T cells and can treat psoriasis [65]. The authors noted that some proteins, such as protein kinase C  $\theta$  (PKC $\theta$ ), that regulate T-cell function are sensitive to fumarate [65]. In a more recent study, Kornberg et al. reported that increased fumarate levels could lead to the negative regulation of glycolysis [66] in a process involving the succination of the active site cysteine (C152) in GAPDH. Research has also shown that endogenous fumarate can succinate GAPDH in both human and mouse macrophages [65,67,68]. The results indicate that increased fumarate levels limit inflammation by acting as an anti-inflammatory signal.

A review of recent studies showed that citrate and ATP-citrate lyase (ACL) are other sources of acetyl-CoA during histone acetylation [65,66]. As shown in Table 2, research indicates that acetyl-CoA is an important cellular signaling metabolite that is derived from citrate during the Krebs cycle [67–69]. Acetyl-CoA is usually generated during the breakdown of proteins, fatty acids, and carbohydrates via amino acid degradation,  $\beta$ -oxidation, and glycolysis [69]. Acetyl-CoA acts as a building block that facilitates the synthesis of amino acids, ketone bodies, and lipids. These functions occur after acetyl-CoA leaves the mitochondria and is converted to ACL; thus, acetyl-CoA plays a signaling role in the context of immune cell biology through ACL. Recent studies further revealed that citrate is a critical element of the Krebs cycle as a source of acetyl-CoA that promotes histone acetylation [38,70,71]. Moreover, citrate can affect immune cell function via a wide range of mechanisms, such as histone modification and acetylation [38,70,71].

**Table 1.** Summary of the 20 main studies.

Study	Methods	Results
Jochmanova and Pacak (2016) [11]	Review	Dysregulated metabolism is one of the primary features of cancer cells, which switch from oxidative phosphorylation to aerobic glycolysis. The metabolic changes also lead to the activation of signaling molecules that support cell survival and proliferation.
Sawa et al. (2014) [14]	Review	Krebs cycle intermediates tend to function as energy substrates in the mitochondrial matrix. In addition, these intermediates have an oxidative impact on the thyroid gland and brain.
Murphy and O'Neill (2018) [16]	Review	Succinate is implicated in hypoxic, metabolic, and inflammatory signaling processes. Moreover, itaconate generated in the Krebs cycle is involved in anti-inflammatory responses.
Desideri, Vegliante, and Ciriolo (2015) [18]	Clinical trial	The TCA cycle is important for oxidative metabolism; it leads to the production of NADH and FADH <sub>2</sub> , thus fueling the mitochondrial electron transport chain and facilitating the synthesis of ATP.

Table 1. Cont.

Study	Methods	Results
Sinton, Hay, and Drake (2019) [19]	Clinical trial	TCA cycle intermediates (alpha-ketoglutarate, fumarate, and succinate) act as allosteric regulators of metabolic enzymes such as the alpha-ketoglutarate-dependent dioxygenase family of enzymes. These enzymes usually target different molecules, such as DNA and chromatin, and are thus involved in modulating gene transcription in response to intracellular lipid accumulation.
Lanni, Moreno, and Goglia (2016) [20]	Review	Thyroid hormones are important endocrine regulators that influence metabolic rates and cellular energy synthesis.
Incerpi et al. (2018) [21]	Clinical review	Thyroid hormone action starts at receptors in the cytoplasm, mitochondria, and plasma membrane.
Conley (2016) [24]	Review	Mitochondria can oxidize substrates during the synthesis of ATP to support different body functions, such as muscle contraction.
Zhao et al. (2019) [29]	Review	The mitochondrial electron transport chain is usually characterized by a proton gradient across the inner membrane and the accumulation of ATP synthase, which facilitate ATP synthesis.
Khatiwada et al. (2016) [35]	Cross-sectional study	The researchers reported thyroid dysfunction in 31.9 percent of patients suffering from metabolic syndrome. The primary forms of thyroid dysfunction were subclinical dysfunction and subclinical hyperthyroidism.
Ryan et al. (2019) [38]	Review	Metabolic reprogramming can be used as the basis for exploring the development of disorders such as diabetes and cancer. In addition, the metabolites involved in these processes have been implicated in the development of immunity in transformed cells.
Meiser et al. (2015) [58]	Clinical trial	Pyruvate dehydrogenase sustains pyruvate oxidation and supports itaconate synthesis. In addition, pyruvate affects immune function by influencing the expression of cytokines.
Lampropoulou et al. (2016) [59]	Animal model study	Itaconate-treated bone-marrow-derived macrophages inhibit the proinflammatory activities of NO, ROS, and the cytokines IL-6, IL-12p70, and IL-1 $\beta$ [37]. In addition, itaconate can hinder the oxidation of succinate by SDH.
Mills et al. (2018) [60]	Animal model study	Itaconate is instrumental in the activation of the anti-inflammatory transcription factor Nrf2 by lipopolysaccharide chains. Additionally, itaconate can modify immune proteins through the alkylation of cysteine residues.
Nemeth et al. (2015) [62]	Animal model study	Itaconate administration can reverse the action of the ADP/ATP translocase and impair SLP. Furthermore, malonate can yield higher levels of ADP-induced depolarization than itaconate.
Arts et al. (2016) [64]	Animal model study	Fumarate accumulation in monocytes improves immune training, enhances cytokine secretion, and inhibits the activity of KDM5 on histones in the immune system.
Blewett et al. (2016) [65]	Clinical trial	DMF inhibits the activation of MMF in cells and regulates the function of T cells through PKC $\theta$ activity.
Kornberg et al. (2018) [66]	Animal model study	Increased fumarate levels lead to the negative regulation of glycolysis. Furthermore, fumarate can succinate GAPDH in macrophages.
Kelly (2015) [67]	Review	Citrate secreted in the Krebs cycle can affect the function of macrophages and dendritic cells. Moreover, succinate can activate the transcription factor HIF-1 $\alpha$ and promote the expression of inflammatory genes.
Chinopoulos (2015) [70]	Review	Succinate formation via the NAD <sup>+</sup> fumarate reductase system can lead to the synthesis of ATP in patients suffering from hypoxia.



**Table 2.** Comparison of the impact of Krebs cycle intermediates on the endocrine system and the immune system.

Impact of Krebs Cycle Intermediates on the Endocrine System	
Citrate	In mitochondria, citrate can hinder the activity of SDH and PDH. In addition, mitochondrial citrate can block fatty acid oxidation by preventing the actions of CPT1. In other cases, citrate promotes fatty acid synthesis by activating the gluconeogenic enzyme FBPase1.
Succinate	Succinate is involved in hypoxia and metabolism and thus influences ATP synthesis and aerobic glycolysis [16]. Succinate provides connections between fatty acid metabolism, carbohydrate metabolism, and epigenetic reprogramming.
NADH	NADH fuels the mitochondrial electron transport chain and affects ATP synthesis [18]. Excessive NADH secretion can lead to a breakdown in the redox balance with $\text{NAD}^+$ , resulting in metabolic syndromes and oxidative stress [18].
Alpha-ketoglutarate	This intermediate functions as an allosteric regulator of metabolic enzymes such as the alpha-ketoglutarate-dependent dioxygenase family of enzymes [19]. Moreover, alpha-ketoglutarate is a critical intermediate in the Krebs cycle that affects the rate of this cycle in the body. Research also shows that alpha-ketoglutarate can stimulate protein synthesis and prevent protein degradation within muscles [19].
Fumarate	Fumarate affects the function of metabolic enzymes that are involved in modulating gene transcription in response to intracellular lipid accumulation [19]. In addition, fumarate modulates nonreductive metabolic pathways such as glycolysis and glutaminolysis.
Impact of Krebs Cycle Intermediates on the Immune System	
Intermediate	Impact
Succinate	Succinate is implicated in immune signaling and the immune response. Succinate accumulation in immune cells can result in signaling via its receptors and in HIF1 $\alpha$ stabilization [55]. Increased succinate levels can inhibit the activity of the prolyl hydroxylase domain of HIF1 $\alpha$ and prevent its stabilization [5]. Finally, succinate hinders HIF1 $\alpha$ ubiquitination and targeting for proteasomal degradation.
Pyruvate	Pyruvate is a Krebs cycle intermediate that affects immune function and the immune response by regulating cytokine expression [58]. In addition, pyruvate increases the uptake of glucose in activated immune cells.
Itaconate	Itaconate promotes and supports the proinflammatory activities of NO, ROS, IL-6, IL-1 $\beta$ , IL-12p70, and other cytokines. Moreover, it prevents the oxidation of SDH [59]. Other studies have shown that itaconate can activate the inflammatory transcription factor Nrf2 and modify immune proteins through the alkylation of cysteine residues [60]. Finally, studies have shown that itaconate can reverse the activity of the ADP/ATP translocase and impair SLP [62].
Fumarate	The accumulation of fumarate in monocytes enhances immune training, improves cytokine secretion, and inhibits KDM5 activity [64]. DMF can also regulate T-cell function via PKC $\theta$ [65]. In addition, increased levels of fumarate lead to the negative regulation of glycolysis [66].
Citrate	Citrate affects the immune system by modulating the function of macrophages and dendritic cells [67]. Furthermore, it can activate the transcription factor HIF1 $\alpha$ and support inflammatory gene expression. Citrate activates TNF $\alpha$ - and IFN $\gamma$ -stimulated macrophages [5]. Finally, the breakdown of mitochondrial citrate is associated with the increased secretion of macrophage inflammatory mediators, including NO, prostaglandin E2, and ROS [5].
Itaconate	Itaconate can reverse the activity of the ADP/ATP translocase, thus affecting the immune response and ATP synthesis for cellular use [70]. Recent studies have shown that itaconate can inhibit SDH and decrease the rate of IL-1 $\beta$ , NO, IL-18, and HIF1 $\alpha$ production [5].

## 6. Discussion

The Krebs cycle is a critical cellular metabolic process. Cells must metabolize glucose to obtain the energy required for growth and other cellular functions [14,15]. Energy can also be used to synthesize the organic molecules required by the body for a wide range of functions, such as movement, digestion, and defense against disease-causing pathogens [16,70,71]. During the metabolism of glucose molecules, vital variables such as heartbeat, respiratory rate, and temperature must be kept stable [12,13,71]; hormones,

enzymes, and metabolic molecules are required to ensure that the body continues to function optimally and within safe limits [21,72,73]. Based on such findings, there have been further investigations to examine the link between the Krebs cycle and critical body systems [8,10,11]. The primary goal is to understand the molecular basis of this relationship and to identify the vital factors that may be targeted to improve health outcomes and manage diseases [74,75].

In recent years, researchers have explored the possible link between metabolism and the endocrine system by examining the effect of Krebs cycle intermediates on various signaling processes [74]. Recent research shows that intermediates such as pyruvic acid, ATP, NADH, and FADH<sub>2</sub> may influence how the endocrine system regulates vital body processes [74,75]. More recently, it was reported that the accumulation or depletion of intermediates as a result of the metabolic syndrome might increase the risk of thyroid dysfunction [76,77]. Thyroid hormones are synthesized by the thyroid gland and are essential for the normal development and function of the body. The secretion of thyroid hormones is a complex process that involves several steps. The first step is the uptake of iodine by the thyroid gland from the bloodstream. Iodine is then oxidized to iodide, which is transported into thyroid follicular cells. The next step is the synthesis of thyroglobulin (TG), a large protein that is the precursor of thyroid hormones. TG is then transported to the colloid of the thyroid follicle, where it is iodinated to form monoiodotyrosine (MIT) and diiodotyrosine (DIT). The iodinated TG is then endocytosed into follicular cells, where it is proteolytically cleaved to release thyroxine (T4) and triiodothyronine (T3). T4 is the major product of the thyroid gland, but most of the T3 is produced by the peripheral conversion of T4.

Metabolic syndrome affects thyroid function at several levels. First, insulin resistance, which is a hallmark of metabolic syndrome, can impair the uptake of iodine by the thyroid gland, leading to reduced synthesis of thyroid hormones. Insulin resistance can also decrease the expression of the sodium iodide symporter (NIS), which is the transporter responsible for the uptake of iodine by thyroid follicular cells.

Second, dyslipidemia, which is also a common feature of metabolic syndrome, can affect the metabolism of thyroid hormones. Low-density lipoprotein (LDL) cholesterol can inhibit the activity of hepatic deiodinases, which are the enzymes responsible for the conversion of T4 to T3 (Table 3). This inhibition can result in a decrease in the peripheral conversion of T4 to T3 and a decrease in the bioavailability of T3.

**Table 3.** Summary of the pathways through which the accumulation or depletion of intermediates due to metabolic syndrome causes disease and their impacts.

	Pathway	Impact
1	Synthesis of thyroid hormones	Impaired uptake of iodine by thyroid follicular cells. Decreased expression of sodium iodide symporter (NIS)
2	Uptake of iodine by thyroid follicular cells	Inhibition of deiodinases by LDL cholesterol and ROS
3	Synthesis of thyroid globulin (TG)	Inhibition of thyroid hormone receptors and deiodinases by cytokines (IL-6, TNF- $\alpha$ )
4	Iodation of TG in colloids	Impact on T3/T4 ratio and T3, T4 levels
5	Endocytosis of TG into follicular cells	Impact on T3/T4 ratio and T3, T4 levels
6	Proteolysis of TG to release T3 and T4	Impact on T3/T4 ratio and T3, T4 levels
7	Peripheral conversion of T4 to T3 by hepatic deiodinases	Impact on T3/T4 ratio and T3, T4 levels

Third, oxidative stress, which is increased in metabolic syndrome, can affect the synthesis and metabolism of thyroid hormones. Reactive oxygen species (ROS) can damage the TG molecule, leading to a decrease in its iodination and proteolysis. ROS can also

inhibit the activity of hepatic deiodinases, leading to a decrease in the peripheral conversion of T4 to T3.

Fourth, inflammation, which is a key feature of metabolic syndrome, can affect thyroid function by increasing the expression of cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha). These cytokines can inhibit the expression of thyroid hormone receptors and the activity of hepatic deiodinases, leading to a decrease in the bioavailability of thyroid hormones.

Therefore, it should be noted that metabolic syndrome can affect thyroid function at several levels, including the uptake of iodine, the expression of transporters and enzymes involved in the metabolism of thyroid hormones, and the synthesis and degradation of thyroid hormones. Such effects can lead to an increased risk of thyroid dysfunction, including hypothyroidism and subclinical hypothyroidism. The interaction between metabolic syndrome and thyroid dysfunction is complex and multifactorial and involves genetic and environmental factors. Understanding the underlying pathways can help in identifying the possible targets for the development of new therapies for diseases such as thyroid dysfunction.

There is sufficient evidence that Krebs cycle intermediates can affect the function and memory of immune cells such as macrophages [68,69,78]. Moreover, these intermediates are directly or indirectly involved in regulating the function and activation of immune cells [79–81]. Thus, targeting such metabolites may help generate new types of therapies [82,83]. For instance, attempts have been made to explore how targeting succinate during an ischemic attack and then targeting the subsequent oxidation of molecules can help manage ischemia-reperfusion (IR) injury [84–86]. In addition, SDH inhibition in patients with ischemia can prevent succinate accumulation and protect against tissue damage [86–88]. Furthermore, researchers contend that intermediates such as malonate may offer protection during reperfusion, while succinate and fumarate may reduce proinflammatory effects by promoting the generation of ROS [87,88]. Understanding the effects of Krebs cycle intermediates on the endocrine system and the immune system is essential in the context of chronic diseases such as adiposity or obesity and diabetes because it can help identify potential targets for therapeutic intervention. Krebs cycle intermediates, such as citrate, succinate, and fumarate, play important roles in regulating the endocrine and immune systems. For example, citrate inhibits the activity of several enzymes involved in glucose and lipid metabolism, including phosphofructokinase and acetyl-CoA carboxylase, which can lead to the development of metabolic disorders such as diabetes and obesity. In addition, succinate and fumarate have been shown to play a role in immunity by activating respective cells and promoting the production of proinflammatory cytokines. Such chemicals can cause chronic inflammation, a key contributor to the development of chronic diseases such as diabetes and obesity. Therefore, knowledge of the effects of Krebs cycle intermediates on the endocrine and immune systems can provide insights into the mechanisms underlying the development of chronic diseases and can help in the development of potential therapeutic targets. For example, targeting the enzymes involved in the metabolism of Krebs cycle intermediates or using drugs that modulate the activity of immune cells could potentially be used as a treatment strategy for chronic diseases.

## 7. Conclusions

In recent years, studies have explored the role of metabolites in signaling processes. The current study entailed reviewing how Krebs cycle intermediates influence the endocrine and immune systems. The results show that different intermediates have unique impacts on various endocrine and immune functions, processes, and pathways. This review highlights the scarcity of data from in vivo and human studies on the epigenetic role of Krebs cycle intermediates. Further research is needed to explore the molecular pathways underlying the actions of Krebs cycle intermediates. The results of such research can be used to develop novel strategies for managing disease by controlling the levels and activity of particular metabolites.

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