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The Use of Echinacea to Improve Oxygen Transport Capacity

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Summary

It is well established that an increase in circulating EPO can result in an increase in oxygen transport capacity and that an increase in oxygen transport capacity may result in an increase in the ability to perform endurance exercise. Echinacea has been used therapeutically in both oral and injected forms and is purported to enhance immune status through an increase in the activity of and/or the circulating concentration of components of the immune system.There is evidence that suggests Echinacea may stimulate the production of EPO and influence erythropoiesis through two separate mechanisms: 1) the activation of macrophages and subsequent production and release of PGE_2 and 2) by increasing the activity level of T cells and subsequent production and release of erythroid progenitor growth factors. An increase in the circulating concentration of PGE_2 is postulated to result in and increase in EPO production directly and indirectly through stimulation of CD4 and CD8 production from T cells. Evidence also exists to support the role of direct stimulation of T cells by Echinacea that may result in production and release of GM-CSF and IL-3. Thus, if Echinacea supplementation were shown to stimulate EPO production then an increase in [RBC] and oxygen transport capacity might also result. Finally, an increase in oxygen transport capacity with Echinacea supplementation may provide a therapeutic means by which to diminish the adverse effects caused by a decrease in oxygen availability and enhance endurance exercise performance through the same mechanism.

Introduction

Clinical conditions such as congestive heart failure, anemia, chronic obstructive pulmonary disease and medical treatments such as chemotherapy can result in a decrease in the amount of oxygen available to working muscle or other tissues of the body. Since oxygen is ultimately required for the conversion of chemical energy to mechanical energy in order to meet the needs of the human body, an increase in the quantity of available oxygen can offset the deleterious effects of certain diseases. Echinacea, an herbal supplement derived from the North American Purple Coneflower plant has been shown to improve oxygen transport capacity in animal models [1]. The oxygen carrying capacity of blood is evaluated by a combination of several variables: 1) the number of red blood cells (RBCs), 2) the concentration of RBCs, 3) hemoglobin (Hb) concentration, and 4) hematocrit (Hct). Erythropoiesis is production of red blood cells and changes in the number of red blood cells; concentration of red blood cells, hemoglobin concentration and hematocrit can be used to indicate erythropoietic activity level. Echinacea supplementation has been associated an increase in erythropoietic factors and erythropoiesis. Evidence to support this assertion is an observed increase in the circulating concentration of erythropoietin (EPO), number of RBCs, the size of RBCs, hemoglobin concentration, and hematocrit [1,2].

Research has also shown that oral Echinacea supplementation in humans resulted in an increase in maximal oxygen consumption (VO_{2max}) [3]. One explanation to account for the Echinacea-induced increase in VO_{2max} is an increase erythropoiesis that results in erythrocythemia as a consequence of an increase in the circulating concentration of the hormone EPO. Erythrocythemiacan result in an increase in oxygen transport capacity, improve exercise economy and $VO2_{max}$ [4-8].

RBCs are derived from stem cells induced by several growth factors including interleukin-3 (IL-3), granulocyte-macrophage colony stimulating factor (GM-CSF) and EPO. Erythropoietin is a hormone that is secreted from the kidney and is considered to be a primary stimulant of erythropoiesis by promoting the formation of and release of RBCs from the bone marrow [9]. Red blood cell production is thought to be regulated within narrow limits so that an adequate number of RBCs is maintained in order to provide sufficient tissue oxygenation and not become so concentrated that blood flow is impeded [10]. The accepted view of the control of erythropoietin production dictates that any condition causing the quantity of oxygen transported to the tissues to decrease results in an increase in circulating EPO and subsequent RBC production [10]. This view was founded on observations made in a variety of disorders and the control of EPO production in healthy individuals may not be quite the same [11]. Research indicates that another protein, prostaglandin E_2 (PGE_2), appears to stimulate production of EPO [12-15]. Echinacea has been shown to stimulate macrophage activity which in turn can result in an increase in PGE_2 secretion from active macrophages [16-21]. Additionally, an increased concentration of PGE_2 has been shown to increase GM-CSF production which is also a erythroid progenitor growth factor. Further, Echinaceasupplementation has been shown to increase the activity level of T cells which are known to synthesize GM-CSF and the erythroid progenitor growth factor IL-3 [21,23-27].These results suggest Echinacea may enhance EPO production and that the mechanism may be mediated through an increase in the circulating concentrations of PGE_2, IL-3, and GM-CSF.

To date, minimal research has been performed to evaluate the effects of oral Echinacea supplementation on EPO production, erythropoiesis or exercise performance. Consequently, the purpose of this review of literature is to provide relevant supporting information and describe the results of previous research.

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Oxygen Transport and Delivery

Components of human blood

The functions of blood in the human body include: 1) transport of dissolved and chemically bound molecules, 2) transport of heat for heating and cooling, 3) transport of signaling molecules, 4) buffering the body fluids, and 5) immune function. The blood volume of the adult human comprises approximately 6-8% of total body mass and 1 L of blood contains approximately 0.46 L of red blood cells (RBCs) in males and 0.41 L of RBCs in females, respectively [28]. This value is typically expressed as the percentage of total blood volume that is composed of formed elements and is referred to as Hct. The fluid portion of blood is referred to as the plasma, and has an osmolality of approximately 290 mosm·kg H_2O^{-1} and contains approximately 65-80 g protein·L^{-1} [29]. The various proteins in the plasma include albumin (57%), α_1-globulin (4%), α_2-globulin (8%), β-globulin (12%), γ-globulin (16%), and fibrinogen (3%) [30]. When blood clots, fibrinogen is incorporated into the clot and the remaining fluid portion is referred to as serum.

Oxygen exchange

Oxygen and carbon dioxide move between the alveolar air and blood by diffusion from an area of higher to lower partial pressure. The partial pressure is the pressure that is exerted by a specific gas within a mixture of gases. At sea level the total pressure of gases in the atmosphere is equal to 760 mmHg with oxygen being 20.93%, carbon dioxide 0.03%, and nitrogen 79.04% of the total. The partial pressure of a gas is calculated by multiplying the total atmospheric pressure by the fraction of percentage of the atmosphere that is represented by a particular gas. Therefore, at sea level oxygen has atmospheric partial pressure of 159 mmHg (PO_2). The PO_2 decreases to 105 mmHg because the gas is warmed and humidified in the conducting zones of the pulmonary system during inhalation and the greater partial pressure of CO_2 in the lungs. The PO_2 of the blood in the pulmonary capillaries is approximately 40 mmHg which facilitates the diffusion of O_2 from the alveoli to the blood due to the PO_2 gradient.

Oxygen transport

Red blood cells are responsible for approximately 97% of the total amount of O_2 that is transported by the blood. The remainder of O_2 that is transported in the blood is dissolved in the plasma.

Red blood cells: A primary function of the RBC is to transport oxygen. Normal RBCs are biconcave discs having a mean diameter of about 7.8 μm and a thickness of approximately 2.5 μm at the thickest point in the center, while the average volume of a RBC is approximately 90-95 μm³ [10]. The bag-like shape of RBCs allows the cells to change shape as they pass through capillaries. Normal RBCs also possess a great excess of cell membrane relative to the quantity of material inside and deformation does not stretch the membrane greatly or result in cell rupture.

Indices that are commonly used to evaluate RBC status include RBC count, mean corpuscular volume (MCV) and red blood cell distribution width (RDW). The RBC count is a measure of the number of RBCs that are contained in one mm³ of peripheral venous blood. The average number of RBCs is 4.7-6.1x10⁶/mm³ males, and 4.2-5.4 x10⁶/mm³ in females [30]. The MCV is a measure of the average volume of a single RBC. MCV is derived by dividing the Hct by the total RBC count. The normal value for MCV is approximately 80-95 μm³ and is considered to be normocytic [30]. When the value for MCV

is abnormally increased the RBC is considered macrocytic, conversely when the value is abnormally decreased the RBC is microcytic. RDW is an indication of the variation in RBC size and is calculated using MCV and RBC.

Hemoglobin: Human Hb is an iron containing pigmented globular protein that is made up of two chains which combine from four different polypeptide chains formed in varying ratios during the different periods of the life cycle. Iron is required for Hb formation because each of the four chains has a porphyrinheme prosthetic group that contains a central ferrous iron atom. There are four ferrous iron atoms in each Hb molecule and each of these can reversibly bind with one molecule of O_2. Combined, these heme groups can transport a total of four molecules of O_2 for each molecule of Hb. During the human life span the following variants of Hb molecules are formed: embryonic Gower 1 and 2, fetal F and two adult hemoglobins A and A_2 [28]. Adult Hb A consists of two α- and two β-chains and is the main component of red blood cells in adults, while adult Hb A_2 accounts for only 2% of the total Hb [28]. The normal molecular weight for the Hb is between 64-69 kilodaltons. When Hb is free in plasma, about 3% leaks through the capillary membrane into the tissue spaces or through the glomerular membrane of the kidney into the glomerular filtrate each time the blood passes through the capillaries [10]. Therefore, for Hb to remain in the blood it must exist inside the RBCs.

Indices used to evaluate Hb status of RBCs include Hb, mean corpuscular Hb (MCH), and mean corpuscular Hb concentration (MCHC). Mean Hb content in peripheral blood is 14-18 g/dL and 12-16 g/dL in males and females, respectively [30]. One RBC contains approximately 27-31 pgHb which is referred to as mean corpuscular hemoglobin (MCH) [30]. Mean corpuscular Hb is a measure of the average amount of Hb within an RBC and is derived by dividing the total Hb concentration ([Hb]) by the RBC concentration ([RBC]). Mean corpuscular Hb concentration is a measure of the average percentage of Hb within a single RBC [30]. Mean corpuscular Hb concentration is derived by dividing the total by the Hct [30].

Red blood cells have the ability to concentrate Hb in the cell fluid up to 34 g/dL of cells [10]. The intracellular Hb concentration ([Hb]) never rises above this value because this is a metabolic limit of the cell's Hb-forming mechanism. Normally the percentage of Hb is almost always near the maximum in RBCs; however, if Hb formation is deficient in the bone marrow, the percentage of Hb in RBCs may decrease considerably below normal. When the Hct is within the normal range 40-45% and the quantity of Hb in each respective cell is normal, the whole blood contains 136-172 and 120-150 g/L of Hb in males and females, respectively [31]. Each gram of Hb is capable of reversibly combining with 1.39 mL of O_2, and under normal circumstances approximately 22 and 19 mL of O_2 can be transported in combination with Hb (oxyhemoglobin) in each dL of blood in males and females, respectively [10].

Dissolved: Approximately 0.17 mL of O_2 per dL of blood is transported by the plasma in a dissolved state [10]. This represents approximately 3% of the total O_2 transport capacity of the blood.

Erythropoiesis

Production of red blood cells: In adults, RBCs are produced by the marrow of the long bones, except for the proximal portions of the humeri and tibiae, which become quite fatty and stop producing RBCs after about 20 years of age [10]. After 20 years of age, most

RBCs are produced in the marrow of the membranous bones, such as the vertebrae, sternum, ribs and ilia. However, it must be noted that marrow production of RBCs decreases in all bones as age increases.

Stimulation of erythropoiesis : The total mass of RBCs in the circulatory system is regulated within narrow limits in order to maintain sufficient tissue oxygenation and to prevent deleterious effects of severe hemoconcentration. Any condition that causes the quantity of O_2 transported in the blood to decrease ordinarily increases the rate of RBC production. The principle factor that stimulates RBC production is a circulating hormone named erythropoietin (EPO). Erythropoietin is a glycoprotein with a molecular weight of approximately 34,000 daltons [10]. In the normal individual, about 90% of all EPO is secreted from the kidneys; the remainder being formed mainly in the liver [10]. In response to hypoxia, EPO begins to be formed within minutes to hours and reaches maximal production within 24-hr of the stimulus; however, new RBCs do not appear in the circulation until after about 5-d [10].

Differentiation of stem cell: Stem cells produced in the bone marrow and identified as pluripotent stem cells are the origin of all circulating blood cells. When pluripotent stem cells are produced a small portion remains undifferentiated in the bone marrow in order to maintain a reserve colony. The majority of pluripotent stem cells differentiate to form the committed stem cells that become RBCs or one of the specific varieties of white blood cells (granulocytes, monocytes, macrophages, megakaryocytes, platelets, T-lymphocytes and B-lymphocytes). The committed stem cells that form RBCs are referred to as erythroid progenitors. There are nine primary lineage stages in the development of RBCs that are identified by sequence of development as follows: pluripotent stem cells, myeloid stem cells, CFU GEMM, CFU-E/MEG/b, BFU-E, CFU-E, normoblast, reticulocyte, and mature erythrocyte [32].The first generations of these cells contain very little Hb but in succeeding generations Hb increases to a concentration of about 34% [10]. At this stage of development the cells no longer contain a nucleus or an endoplasmic reticulum, and are called reticulocytes [10]. During the reticulocyte stage, the cells still contain some of the basophilic material and move from the bone marrow into the blood capillaries. The remaining basophilic material usually disappears within 1-2 days and the cell is then considered to be a mature erythrocyte [10].

Regulation of erythropoiesis: Growth and differentiation of pluripotent stem cells is regulated by growth factors that exert effects on the proliferation, differentiation, and functional activation of both progenitors and mature cells [33]. The growth factors known to be involved in erythropoiesis are EPO and cytokines produced by monocytes, macrophages, T-lymphocytes, endothelial cells, and fibroblasts including GM-CSF, granulocyte colony-stimulating factor (G-CSF), interlukins-1, 3, 4, 6, 9 and 11, stem cell factor (SCF), and insulin growth factor-1 (IGF-1) [32,33]. Erythropoietin acts in the terminal stages of RBC development from erythroid progenitor cells and primarily on CFU-E [32]. Erythropoietin acts synergistically with SCF, GM-CSF, IL-3, IL-4, IL-9, and IGF-1 to cause maturation and proliferation from the stage of BFU-E and CFU-E to the normoblast stage of erythroid progenitor development [34,35]. Thus, EPO is thought to function primarily to decrease the incidence rate of apoptosis in erythroid progenitor cells [32]. Stem cell factor, IL-1, IL-3, IL-6 and IL-11 provide the stimulus to cause differentiation of the pluripotent stem cell into the myeloid stem cell and the CFU-GEMM

[32]. The CFU-GEMM gives rise to specific CFU for granulocytes, monocytes, megakaryocytes, macrophages, eosinophils, and erythroid cell precursors [36-38].

Iron metabolism: Iron is a metal that exists in two oxidation states in the human body, ferrous (Fe (II)) and ferric (Fe (III)). The iron contained in Hb is in the Fe (II) oxidation state has the ability to combine with and transport O_2, whereas Fe (III) iron does not. The life of a RBC is approximately 120 days and once the membrane loses integrity and becomes permeable to ions, the iron transforms to the Fe (III) and the Hb becomes known as methehemoglobin. The amount of iron in the blood 14-32 and 11-29 μmol/L in males and females, respectively [31]. The total quantity of iron in the body averages 4-5 grams, 60-70% of which is contained in Hb, 10-12% contained in myoglobin, 16-29% stored in the form of ferritin and hemosiderin [29].

Iron from the diet is absorbed in the small intestine and immediately combines with apotransferrin to form transferrin which is then transported in the blood plasma. The iron is loosely combined with the globulin molecule and can be released to any body tissues. Excess iron in the blood is deposited in all cells but especially in the liver hepatocytes and to a lesser degree to reticuloendothelial cells of the bone marrow. In the cell cytoplasm, iron combines mainly with apoferrittin to from ferritin. Varying quantities of iron can combine in clusters of iron radicals in apoferrittin, and when iron is stored as ferritin it is called storage iron. Less significant quantities of iron are stored in an extremely insoluble form called hemosiderm. The storage potential of hemosiderm becomes important when the total quantity of iron in the body exceeds the storage capacity of ferritin. When the quantity of iron in the plasma falls below the homeostatic set point, iron is removed primarily from ferritin and is then transported as transferrin. A unique characteristic of the transferrin molecule is that it binds strongly with receptors on the cell membranes of erythroblasts in the bone marrow where it is ingested by endocytosis. After entering the erythroblast, transferrin can deliver iron directly to the mitochondria where heme is synthesized. After the life cycle of a red blood cell is complete, the hemoglobin released from the cells is ingested by the cells of the monocyte-macrophage system and the iron is then stored [10].

Erythrocythemia

Erythrocythemia is the term used to denote the state of having an increase in total red cell mass of the blood. In this state the RBC count can increase to 6-7x10^6/mm³ RBCs [10]. Other terms that are used to denote this state include polycythemia, hypercythemia and hypererythrocythemia. Erythrocythemia can be the result a disease state, tissue hypoxia such as when there is a lack of availability of oxygen in the atmosphere, and may be induced by infusion or pharmacologic means.

Induced erythrocythemia: Induced erythrocythemia, blood doping, blood boostingareterms used to describe the procedure of increasing [RBC], the consequence of which is an increase in [Hb] to levels that are above normal in an attempt to improve endurance exercise performance. This state may be achieved through a number of methods that include: 1) the infusion of RBCs from a donor, 2) the removal and subsequent infusion of one's own RBCs, and 3) the use of recombinant human erythropoietin (r-EPO).

RBC infusion can be done through autologous or homologous transfusion. Autologous transfusion involves withdrawing 450-1800 mL of whole blood from an individual removing the RBCs and reinfusing the plasma component. The RBCs that are removed are

stored in either refrigerated of frozen state for later use. Homologous transfusion involves infusion of blood from a type-matched donor. In order to prevent dramatic reductions in RBC concentration when performing autologous transfusion the withdrawal of blood from the individual occurs over a 3-8 week time period, which approximates the time required to reestablish normal RBC count and [Hb] after RBC removal [39]. After RBC removal, erythropoiesis is stimulated by the lowered O_2 content of the blood and RBCs are produced to replace the quantity that was lost.

Blood can be preserved by refrigeration at 4°C for 3 weeks with an expected 60% viability rate of RBCs [6]. Refrigeration is the primary method by which blood is normally stored for blood banks. There is an expected loss of 1% of the RBC population for each day that the blood is stored; therefore, there may be a progressive loss of erythrocytes approximating 15-20% prior to reinfusion [40]. Additional loss of erythrocytes occurs due to adherence to storage containers, handling losses and increased membrane fragility due to storage [6]. Storage of blood by refrigeration is generally limited to 3-5 weeks by national and international health regulations [5]. Another method by which to preserve RBCs is the high glycerol freezing technique. Using the high glycerol freezing technique the aging process of the RBCs is interrupted and cell membrane integrity is less effected as compared to refrigeration [40]. Further, this technique allows the recovery of 85% of the stored RBCs and blood can be stored indefinitely [5].

Stored RBCs are generally reinfused 1-7 d before endurance competition and has been shown to increase RBC count and [Hb] levels as much as 8-20% [39]. Hemoconcentration translates to an average [Hb] increase for males that may reach 19 g/dL and result in a Hct of approximately 60% [39]. After reinfusion these hematologic parameters may remain in an elevated state but will decline toward control levels over the following 120 days [6].

Recombinant DNA technology has allowed for the cloning of the gene responsible for EPO and has allowed the recombinant version of this hormone (r-EPO) to be synthesized. Recombinant human erythropoietin is injected into the blood stimulating an increase in RBC production and release as previously described. This pharmacologic intervention is used to anemia associated with renal disease and anemia associated with nonmyeloid malignancies where the anemia is due to the effects of concomitantly administered chemotherapy [32,39]. Additionally, r-EPO has also been shown to improve maximal aerobic power [41,42].

Effects of induced erythrocythemia on acute aerobic exercise

An ergogenic effect on VO_{2max} has been shown following the removal of as little as 800 mL of blood due to the resultant decrease in [Hb] and oxygen transport capability [43,44]. In these two studies packed red cells were reinfused after 30-35 d causing an induced erythrocythemia. Further, an ergogenic effect on VO_{2max} was observed with the induced erythrocythemia [43,44].

The first observations of erythrocythemia were noted in reports of observations of native mountain populations that lived at an altitude of 14,000 ft above sea level [45,46]. These observations also documented an above normal [Hb] in these populations [45,46]. Training at altitude has since been shown to improve performance in endurance exercise performed at sea level [45,46].

Induced erythrocythemia has been shown to increase performance during acute aerobic exercise through enhanced O_2 transport and an increased buffering capacity of blood [6,7]. In order for an increase in [Hb] to result in an increase in VO_{2max} several assumptions must be met: 1) maximal cardiac output must not decrease following erythrocythemia due to an increase in blood viscosity, 2) distribution of maximal cardiac output to working muscles must not decrease, 3) muscles must have the ability to extract the additional O_2, and 4) muscles must have the oxidative capacity to utilize the additional O_2 [6].

The first investigation of the effects of induced erythrocythemia cute exercise performance reported that reinfusion of 900 mL blood after 4-weeks increased [Hb] by 13%, increased physical performance as measured run time to exhaustion and increased VO_{2max} 23% in physical education students [43]. Another report indicated that reinfusion of 900 mL of cryo-preserved blood resulted in a significant 9.3% increase in [Hb], a 5.1% increase in VO_{2max} and a 34% increase in treadmill running time to exhaustion in elite runners [8]. An important aspect of this investigation is that blood was not reinfused until hematological parameters returned to pre-blood removal levels as compared to the 4-week period used in the previous investigation. A subsequent investigation examined the effects of infusing 900 and 1,350 mL of cryo-preserved blood elite runners [49]. One purpose of this investigation was to determine whether there was any impairment in cardiovascular function as a result of the induced erythrocythemia. No evidence of impaired cardiac function was noted during light to moderate exercise as the result of reinfusion of either 900 or 1,350 mL of blood. Results indicated a significantly increased Hct (7.9 and 10.8%, respectively) and VO_{2max} (3.9 and 6.6%, respectively) [49]. These findings confirmed the results of the previous investigation and revealed that the VO_{2max} was improved due to an increase in the volume of blood reinfused [49]. Further support for the benefit of induced erythrocythemia has been well documented [4,50-52]. These investigations reported significant improvements in aerobic performance and VO_{2max} as a result of the reinfusion of 900 mL or more of cryo-preserved blood.

Other investigations have indicated that r-EPO administration improves aerobic capacity and endurance performance [7,53]. These results were attributed to stimulation of erythropoiesis which leads to an increase in arterial O_2 content. However, the use of r-EPO has also been associated with potentially serious complications including hypertension and thrombolytic or convulsive events [54].

Echinacea

Botany: The term Echinacea is the generic name for a number of widely used plant derived herbal supplements. Although the Echinacea plant is indigenous to North America, it has also been grown commercially in Germany since the 1930's [55] . The Echinacea plant is a member of the *Compositae* family which also includes calendula, chamomile and feverfew [56]. Common names for the Echinacea plant include the purple coneflower, red sunflower, thimbleweed and udbeckia [56]. The Echinacea plant varies in location by species, but is generally found in open meadows or damp locations such as woods, swamps, ditches, river banks and low-lying thickets [56]. There are nine known subspecies of Echinacea, *but* only three *Echinacea angustifolia* , *Echinacea purpurea* and *Echinacea pallida* have been identified as constituents in medicinal preparations [55]. The roots and stems are typically used to produce supplements made from *Echinacea pallida* and *Echinacea angustifolia*. Supplements derived from *Echinacea purpurea* are primarily prepared from fresh leaves, stems and flowers [57].

Echinacea plants are herbaceous perennials that vary in height from 10-60 cm [55]. The stem of the plant ascends either from a vertical

taproot, as in *Echinacea angustifolia* and *pallida*, or from branched, fibrous roots as in *Echinacea Purpurea* [55]. As all members of the *Compositae* plant family, each flower head is actually a conglomeration of many tiny flowers. The inner flowers end in spines and are surrounded by droopy outer flowers with teeth at the ends. Echinacea is characterized by spiny flowering heads, with an elevated receptacle which forms a cone [55]. Colors of the inner flowers range from green to red-brown and the outer flower petals may be pink, white or purple [55]. Echinacea plants are resilient and drought resistant, but grow very slowly [55].

History: *Echinacea angustifolia* was used therapeutically for centuries by Native Americans to treat eye conditions, snake bites, insect stings, infected wounds, eczema, enlarged glands, mumps and rabies [56]. *Echinacea angustifolia* was also used by Native Americans as a painkiller for a variety of conditions from stomach aches to epilepsy [56]. A paste made of the entire mashed plant was used to treat snake bites, insect stings, burns and swelling of lymph glands [55]. The roots were chewed or the juice was ingested to treat sore gums, toothaches and sore throats [58]. European colonists soon began to use Echinacea and to send the plant back to the European markets [55]. In the 1800's *Echinacea angustifolia* was used as an all-purpose "blood purifier" and an anti-infective agent [58]. In the late 1800's, *Echinacea angustifolia* was the most commonly used plant remedy in the United States, however, use began to decline around 1920 due to primarily to the advent of antibiotics [56]. Although *Echinacea angustifolia* use in the United States was declining, demand in Europe was on the rise and eventually became greater than the supply from the United States [55]. As a result, The Madaus Company in Germany imported Echinaceaseeds and began cultivating Echinaceaplants for commercial sale in the European markets [56]. Interestingly, the seeds imported to Europe were *Echinacea purpurea* instead of *Echinacea angustifolia* [55]. As a result of this oversight by The Madaus Company, *Echinacea purpurea* has since become the most widely used of three subspecies of Echinaceaplants. Recently, anecdotal successes and scientific studies have propelled Echinacea to the top ranks of American herbal sales [55]. Presently, Echinacea is primarily used as a non-specific immunostimulant and to prevent upper respiratory infections.

Echinacea supplementation

There are no studies that have directly evaluated the efficacy of Echinaceasupplementation to enhance submaximal exercise economy, maximal aerobic capacity or measures of erythropoietic status. Moreover, no research has investigated whether the proposed Echinacea-induced erythropoiesis is associated with increased concentrations of EPO, IL-3, GM-CSF, or PGE_2. However, when evaluated collectively, data from several investigations provides indirect evidence of the possible existence of these effects [1,21,23-27].

Maximal exercise capacity: The effects of supplementation with either Echinaceaor the active components of a Taiga Wurzelpreparation (*Eleutherococcussenticoscus*) on cellular defense and physical fitness were evaluated in 50 healthy male and female volunteers. *TaigraWurzel* is a variety of commercially prepared Siberian ginseng. The volunteers were divided into two groups with n = 35 in the Taiga Wurzel and n = 20 in the Echinacea group, respectively. Maximal incremental exercise tests were performed on a cycle ergometer prior to and following four weeks of oral supplementation on 20 males that were randomly selected from both groups. The Echinaceagroup was supplemented with 40 drops of 80g of *Echinaceapurpurea* fresh juice in 100g of the final product containing 22% ethanol [3]. Results indicated

that four weeks of oral Echinacea supplementation in humans resulted in a 5% increase in VO_{2max}, 35.03 and 36.91 $mLO_2 \cdot kg^{-1} \cdot min^{-1}$ respectively [3]). It is important to note that the improvement in VO_{2max} was not statistically significant and the effect of Echinaceasupplementation on VO_{2max} was not a specific research question addressed in this investigation. Additionally, no measures of erythropoietic status were evaluated in this investigation.

Echinacea induced erythropoiesis

One explanation to account for the increase in VO_{2max} observed in the previous investigation is the possibility that Echinaceasupplementation may induce an increase in erythropoiesis that results in erythrocythemia due to an increase in the circulating concentration of EPO. Erythrocythemia has been shown to result in an increase in oxygen transport capacity, an improvement in exercise economy, and VO_{2max} [4-8]. Evidence for this explanation can be found in a study conducted to collect data regarding the efficacy of Echinaceasupplementation to stimulate equine immune function. This study was designed as a placebo-controlled, randomized, cross-over trial with n = 6. Horses were supplemented for 42-d with 30 mL of either placebo or Echinacea as a topcoat of their normal food with the cross-over trial following a 12-d washout period.

The supplement used in this investigation consisted of a 1:3 ratio of powdered

Echinacea added to the topcoat of the normal horsefood were supplemented for 42-d with 30 mL of either placebo or Echinacea as a *Echinacea angustifolia* equivalent to 3000mg crude powdered root suspended in a sucrose and water solution to a final concentration of 1000mg standardized extract to for each dose [3]. Blood samples were collected at day 0, 7, 14, 21, 28, 35, and 42 of each trial and analyzed for variables including RBC, Hb, Hct, MCV, MCH, and MCHC. Results indicated that after 21-d of supplementation RBC, Hb, Hct, and MCV were significantly greater in the Echinacea group as compared to the control group [1]. Further, these variables remained significantly greater in the Echinacea group than the control until termination of data collection on day 42 of the study [1]. These data indicate that 21-d of Echinaceasupplementation results in a significant increase in hematological measures of erythropoietic status and that the effects last for a minimum of 42-d in an equine model. It must be noted that the data from this investigation was graphically depicted as percent change and that no actual values were presented in the results or discussion.

Echinacea and erythropoieti: Red blood cell production is thought to be regulated within narrow limits so that an adequate number of RBCs is maintained in order to provide sufficient tissue oxygenation and not become so concentrated that blood flow is impeded [10]. The accepted view of the control of EPO production dictates that any condition causing the quantity of oxygen transported to the tissues to decrease results in an increase in circulating erythropoietin and subsequent RBC production [10]. This perspective was founded on data obtained exclusively from diseased individuals and the control of EPO production in healthy individuals may not be quite the same [11].

There is evidence that suggests Echinacea may enhance the production of EPO and influence erythropoiesis through a mechanism involving the activation of macrophages and subsequent production and release of PGE_2. Prostaglandin E_2 is released by the renal medulla, gastrointestinal mucosa, and activated macrophages [59]. Known functions of PGE_2 include modulation of inflammatory responses, an increase in vascular permeability, an increase in pain sensitivity,

pyrogenic action, suppression of lymphocyte transformation, release of mediators from mast cells, cell-mediated cytotoxicity, renal vasodilation, inhibition of renal tubular resorption and gastric secretion, and tissue dependent contraction and relaxation of smooth muscle [59]. Activated macrophages are known to secrete PGE_2 and Echinacea has been shown to stimulate macrophage activity in mice, rats, and isolated human peripheral blood cells [16-21]. Activated macrophages are also known production sites of other erythroid progenitor growth factors including GM-CSF [32]. Many investigations have demonstrated that PGE_2 enhances the production of EPO [12-15,60,61]. The first investigation to report this finding in human subjects was a clinical trial on four anemic patients with end stage renal disease [13]. Results indicated an increase in EPO and peripheral BFU-E that was shown to return to baseline with the cessation of PGE_2 therapy [13]. Other evidence comes from two investigations performed in vitro with rat renal mesangial cell cultures [14,15]. These results indicated that both EPO and PGE_2 could be produced from rat renal mesangial cell cultures and that EPO production was further enhanced with both and PGE_2 hypoxia [14,15]. The most recent evidence to confirm these findings determined that EPO enhancement by PGE_2 was mediated through a cyclic adenosine monophosphate protein kinase response element binding protein pathway [12]. Therefore, an increase in PGE_2 production from Echinacea stimulated macrophages may enhance renal EPO production.

Echinacea and erythroid progenitor growth factors: Activated T cells are known to synthesize and release GM-CSF and the erythroid progenitor growth factor IL-3 [23,27]. Echinacea supplementation has been shown result in an increase in the activity level of T cells [21]. Evidence to support this role comes from an investigation evaluating the protective effects of *Echinacea purpurea* against radiation through changes in the peripheral blood cell count and peripheral blood antioxidant activity in mice. *Echinacea purpurea* administration exhibited a suppressive effect on radiation-induced leukopenia, especially on lymphocytes and monocytes, and resulted in a faster recovery of blood cell counts [21]. Cytokines released from macrophages in mouse peripheral blood after *Echinacea purpurea* administration activated T cells to proliferate [21]. In addition, the authors concluded that the T cell subsets CD 4 and CD 8 were more enhanced by *Echinacea purpurea* administration than helper T cells or suppressor T cells [21]. These results suggest Echinacea may enhance erythroid progenitor growth factor synthesis.

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