
Investigation on the Mechanism of Qi-Invigoration from a Perspective of Effects of Sijunzi Decoction on Mitochondrial Energy Metabolism

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1. Introduction

Traditional Chinese medicine (TCM) is an ancient Chinese medical system that takes a deep understanding of the laws and patterns of nature and applies them to the human body. TCM, which is also considered as an alternative medicine, is gradually being accepted and is practiced even in the Western world, is the quintessence of the Chinese cultural heritage, has made an everlasting contribution to the survival, propagation and prosperity of all ethnic groups in China, thereby enhancing the fertility and prosperity of the nation. TCM has been practiced by the Chinese for five thousands of years and is rooted in meticulous observation of how nature, the cosmos, and the human body are interacting. Major theories include: Qi, Yin and Yang, the Five Phases (Wu Xing), the human body Meridian system (Jingluo) and viscera and bowels (Zang Fu organs) theory.

Western medicine places strong emphasis on the physical structures of the body, which are made up of different organic and inorganic substances, proteins, cells and tissues. These substances form the physiological basis of humans. Western medicine treats disease at microscopic point of view. TCM, on the other hand, views life differently. Instead of emphasizing discrete body components, the body is seen as a whole entity with connecting parts that work together to sustain life. TCM studies the world from the macroscopic point of view, and its target is to maintain the original harmony of human being [1]. Qi, Blood and Body Fluids are the most important fundamental substances necessary for life. Western Medicine is different from TCM because the TCM has a concept of Qi as a form of energy. It is suggested that Qi was "born" at the same instant as the rest of the universe, and that we are all born from the Qi of the universe. The ancient concepts of Qi are the foundation of TCM and accordingly,

disease or sickness is caused by a disruptive flow of energy or the imbalance of the Yin and Yang energies around our human bodies. Hence, TCM provides a holistic treatment [2]. Qi is said to be the unseen vital force that nourishes one's body and sustains one's life. An individual would become ill or dies if one's Qi in the body is imbalanced or exhausted [1].

The total loss of Qi is what Chinese medicine refers to as death. And here are Chinese people from all walks of life as they seek relief, through a rebalancing of their Qi, their vital energy, for ailments from colds to cancer. The ultimate goal of Tai Chi is to control and direct the Qi within the body. But does Qi really exist? It has no place in Western medical practice, but is essential to the practice of traditional medicine in China. Have ever you had a dramatic spiritual or emotional experience and felt energy literally rushing through your body? I believe this is Qi energy at work, moving through the body. The two types of medical practice existed side by side in China, and had little intercourse with one another. And from the Chinese perspective, Qi is the origin of true strength and power as well as genuine health—body, mind, and spirit.

All kinds of diseases and ailments are born from Qi in TCM, Qi deficiency is the common cause of a variety of diseases and can lead to energy metabolism dysfunction, and Qi-invigoration is the basic principle for treatment of Qi deficiency. Due to the popularity and therapeutic values of "Qi-invigorating" herbal formulae, the investigation of biological activities and the underlying mechanisms in relation to "Qi-invigoration" is of great pharmacological interest. However, the mechanisms for Qi-invigoration in TCM remain elusive. In this regard, our previous studies show that all the four widely used Qi-invigorating herbal medicines (including ginseng, astragalus root, pilose asiabell root, white atractylodes rhizome) can increase bioenergy level of liver cells *in vivo*. We propose a hypothesis that Qi is closely related to bioenergy according to the ancient concept of Qi and modern bioenergetics [3]. The Qi-invigorating representative prescription Sijunzi Decoction (SD) is widely used for treatment of Qi deficiency. However, the pharmacological basis of "Qi-invigorating" action has yet to be established. This chapter aims to provide a comprehensive overview of Qi and Qi-invigoration in TCM, analyze mitochondrial protection and energy metabolic improvement of SD, find its underlying mechanism, thus further reveal the nature of Qi-invigoration in TCM from mitochondrial energy metabolism perspective, help to interpret the concept of Qi scientifically, and provide a new way of thinking and scientific evidence in guiding Qi-invigorating prescriptions for the treatment of energy metabolism- and mitochondria-related diseases. The mechanism of SD on energy metabolism improvement will be explored from mitochondrial oxidative phosphorylation and intracellular adenylates levels.

2. The ancient Chinese philosophy background in formation of Qi concept in TCM

Perhaps the greatest genius of the ancient Chinese sages, and the insight that has given TCM its uninterrupted longevity and effectiveness as a complete medical system, was their discovery of Qi that gives life to everything in the universe and is everything in the

universe at the very root of reality. It's impossible to really understand TCM and its incredible healing power throughout the millennia without realizing the importance of Qi. The unseen power and intelligence that animates and orchestrates all physical and meta-physical processes is Qi. It is Qi that delivers the necessary information and messages between all body structures and systems, and it is Qi that enables us to connect with the natural world and the Universal. Qi is the life force that permeates everything in the universe. Without it there can be no growth and change. Your physical body cannot exist without Qi. When you die your Qi leaves—it's transformed.

The theory of Qi in ancient philosophy was introduced into the medical field, the basic theory of Qi in TCM was formed, i.e., the concept of Qi in TCM was established during the mutual penetration between the materialist philosophy and medicine in ancient China. Qi has been used as a healing technique in China for 4000 years. The origin of the character of Qi was traced back to 3500 years ago. Confucius (who lived approximately 2500 years ago), taught moral and ethical behavior. In his *Analects*, the character of Qi appeared in four locations. It expressed the concept related to breathe, food and vitality.

Taoism, which was founded by Lao-Tze (who was believed to have lived around the time of Confucius or 100 later), have had more influence on Qi and Qigong. In the book "*Zhuangzi*", which compiled the thoughts of Lao-Tze in the third century BC, the character of Qi appeared 39 times. What it explained was: "Qi exists throughout the universe, when Qi assembles, it appears as a human life; when Qi disassembles, the human will die. Therefore, do not worry about life and death. Live naturally and freely as you are". When one studies the principle of the Life Force, the Qi and the Tao (Yin and Yang); one would understand how this Life Force manifests in nature. Through self-cultivation, one basically enriches one's Qi for optimum health and longevity. This happens when one subscribes to this Life Force from nature that flows freely into one's mind and body. However, this requires one to live freely from desires, worries and emotions. To live freely, one has to detach from the worldly possessions. For the instance, money is to be spent, there is to-ing and fro-ing, thus a going and a coming of it; and there is a non-attachment to the money or the material things for better flow. Furthermore, one is required to discipline oneself by having a proper diet, sleep and exercise so that one would not disturb and interrupt with the movement of Life Force which may cause the Qi to dissipate in one's body. This dissipation of Qi would result one to fall into sickness, disease, physical and mental sufferings. It is the Taoist's belief that the practice of Qigong, Tai Chi movements and meditation helps one to harmonize one's Life force with one's environment and nature [2].

"Two classic medical texts, the *Nei Jing* (compiled from 100 B.C. to 100 A.D.) and the *Nan Jing* (written circa 100 to 200 A.D.) were important early documents that presented the core concepts of TCM, and they have informed generations of scholars and practitioners ever since. These core concepts suggest that disease is the result of imbalances in the flow of the body's Qi, and that the human body is a microcosm of the basic natural forces at work in the universe. Generally speaking, Qi is an essential substance that is full of vigor and flows fast. The *Yellow Emperor's Inner Canon (Nei Jing)* teaches us: "It is from calm, indifference, emptiness, and nondesiring that true Qi arises. If the spirit is harboured inside, whence can illness arise? When the will is at rest and wishes little, when the heart is at peace and fears nothing, when the body

labours but does not tire, then Qi flows smoothly from these states, each part follows its desires, and the whole gets everything it seeks'. The Chinese philosopher, Mencius (372–289 BC) described Qi in terms of moral energy, related to human excellence. This reinforces the argument that Qi is contextual, fluid in nature and not a fixed entity.

3. The concept of Qi

Qi is the hub from basic theory to clinical practice and health longevity in TCM. The true foundation of TCM is Qi. Qi, is an important category in the ancient Chinese philosophy, is a simple understanding of natural phenomena. In TCM, Qi is constantly in motion, is the subtle substance with a strong vitality which constitute the human body and maintain the activities of human life, is one of the most basic material, it is also known as the "essence Qi". When the concept Qi in TCM was used to discuss the human body, it often has the meaning of both life material and physiological functions. Therefore, Qi in TCM is one of the most important basic concepts. Qi is the basis for unifying theories of TCM, and Qi theory is the core of the basic theories of TCM. According to TCM, " Qi is fundamental to human, both life and death of human all depend on Qi, if Qi gets together, it will result to the birth; if Qi is harmonious, then the human body is healthy; if Qi decline, the body is weak; if Qi is disordered, the human will be sick; if Qi is depleted, the human will die; therefore, unharmonious Qi is fundamental to the disease." The concept of Qi is complex and messy, connotation of Qi is colorful, extension of Qi is all-pervasive and unlimited, Qi becomes the enigma of Chinese medicine. Because modern medicine has not the concept of "Qi", Qi is the biggest difference between Chinese and Western medicine, which caused communication barriers between the two systems of medicine.

3.1. The meanings of Qi

What is Qi? The concept of Qi is based on the ancient Chinese initial understanding of natural phenomena. That is, Qi is the most basic substance of which the world is comprised. Everything in the universe results from the movements and changes of Qi. This concept was introduced into TCM and became one of its characteristics. The meaning of Qi in TCM has two aspects. One refers to the vital substances comprising the human body and maintaining its life activities, such as the Qi of water and food (food essence), the Qi of breathing (breathing nutrients) and so on. The other refers to the physiological functions of viscera and bowels, Meridian system, such as the Qi of the heart, the lung, the spleen and the stomach and so on.

The ancient Chinese people believed Qi was the most fundamental entity making up the world. The Chinese character for "Qi" is the same word used for air or gas, and it is thought to have the same properties as these substances. While Qi is often described in the West as energy, or vital energy, the term Qi carries a deeper meaning. Qi has two aspects: one is energy, power, or force; the other is conscious intelligence or information. Qi can be interpreted as the "life energy" or "life force," which flows within us. Sometimes, it is known as the "vital energy" of the body. In fact, it may be difficult to find one equivalent English word or phrase that

completely describes the nature of Qi. Most often, Qi is best defined according to its functions and properties. In the human body, Qi flows through meridians, or energy pathways. Twelve major meridians run through the body, and it is over this network that Qi travels through the body and that the body's various organs send messages to one another.

3.2. The sources of Qi

Man depends on nature for his production and growth and must observe the common laws of the world. As everything in the world comes from the interaction of Heaven Qi and Earth Qi, man must breathe to absorb Heaven Qi and eat to absorb Earth Qi. The food Essence transformed and transported by the Spleen¹ must be sent up to the Lung to combine with fresh air to produce the nutrients necessary for man's life activities. Qi of the human body comes from the combination of three kinds of Qi, Primordial Qi inherited from parents, the fresh air inhaled by the Lung and the refined food Essence transformed by the Spleen. Both the inherited and the acquired vital energies are further processed and transformed by the organs. The kidney first sends the innate vital substance upwards where it combines with food essence derived from the spleen. It further mixes with the fresh air from the lungs where it finally forms into Qi of the body.

By understanding how Qi is formed, TCM has identified two important factors necessary for maintaining health. By eating a healthy diet and breathing fresh air, the body extracts their most valuable essences and uses them to help form the vital energy. Following these simple principles are the first steps towards creating a healthy balance in the body. By keeping your daily source of energy—Acquired Qi—strong and balanced, energy is saved because a healthy Spleen and Stomach can extract more Qi from food and drink. Choosing food wisely and eating at regular intervals helps accomplish this.

3.3. The functions of Qi

Generally speaking, Qi of the human body has five functions: promoting, warming, defending, controlling and transforming. Qi provides the active, vital energy necessary for the growth and development of the human body and to perform the physiological functions of the organs, meridians and tissues. In addition, Qi promotes the formation and circulation of blood and

¹ Both TCM and western medicine have the name of "spleen" organ, but connections and differences between them were perceived. TCM practitioners pay more attention to the function than the organ entity in the viscera concept. The Spleen is one of the viscera (zàng) organs stipulated by TCM, it is a functionally defined entity and not equivalent to the anatomical organ of the same name in Western medicine. The Spleen transforms and transports food Essence from the food after it has been preprocessed by the Stomach and the Small Intestine, and then distributes it to the whole body, especially upwards to the Lung and Heart, where food Essence is transformed into Qi and Blood. In this spirit, the Spleen is also called "root of the postnatal". Thus, TCM also describes the Spleen as the source of "production and mutual transformation" of Qi and Blood. The function "the Spleen governs transportation and absorption" and that of the pancreas have many things in common, therefore, the Spleen in TCM should include spleen and pancreas in Western medicine. The Spleen also assists the body's water metabolism, exercises control over the blood inside the vessels and governs muscles and limbs. Whereas spleen is the largest lymphoid organ in the human body in Western medicine, and its main function is to participate in the function of the immune response of the lymphoid tissue and it is closely related to cellular and humoral immunity. The author considers that the core connotation of the Spleen in TCM is energy metabolism, i.e., the process of cellular energy metabolism is the function of the Spleen, where organelles that in charge of the energy metabolism in the cell (i.e., mitochondria) may attribute to the Spleen in TCM.

supports the metabolism of body fluid. If there is a deficiency of Qi, its promoting functions are weakened. As a result, growth and development can be affected or delayed, the organs and meridians cannot function properly and blood formation is hampered, leading to a series of health problems. Qi also contains heat energy for the body. Being a heat source, Qi warms the body and keeps it at a constant temperature so normal physiological functions can take place. Deficiency of Qi can lead to a lowered body temperature, intolerance of cold and cold hands and feet. In TCM, "Evils" are environmental factors that lead to illness. They are classified as wind, summer heat, dampness, dryness, cold and fire. One of the main causes of disease is the invasion of "Evils". By resisting the entry of "illness evils" into the body, Qi defends against their attack and maintains healthy physiological functions.

Qi consolidates and retains the body's substances and organs by holding everything in its proper place. Qi keeps the blood flowing within the vessels and prevents it leaking out, controls and adjusts the secretion and excretion of sweat, urine and saliva, and keeps body fluids from escaping the body, consolidates and stores sperm to prevent premature ejaculation, and consolidates the organs and stops them from descending into a position where they cannot function properly. If Qi is deficient, the consolidating function is weakened, leading to various kinds of health problems such as haemorrhage, frequent urination, premature ejaculation and stomach or kidney prolapses. The promoting and consolidating functions work in a complementary manner. For example, Qi promotes blood circulation and the distribution of body fluids, but it also controls and adjusts the secretion of fluid substances. The balance between these two functions is essential for maintaining a healthy blood circulation and water metabolism. Qi also possesses vaporization or "transformation" functions, which are important for the metabolism of fundamental substances. As suggested by these words, Qi may "vaporize" substances in the body and transform them into essence or vital energy. For example, certain actions of Qi allow food to be changed into food essence, which is in turn transformed into different types of Qi and blood. Indigestible food and waste are also transformed by Qi into urine and stools for excretion.

3.4. The movement of Qi

Qi flows throughout the whole body because of its strength and vigor. The movement of Qi is called Mechanism of Qi, which can be generalized as four aspects: ascending, descending, entering and exiting movements, which are based on directions. Qi was originally a philosophic concept. Through out the ages, the Chinese have developed working constructs which serve to explain the observable phenomenon of the natural world. The idea of Qi is one of the most basic building blocks upon which the Chinese, of both ancient and modern times, conceive the universe. The concept of Qi is a fundamental stratagem in the practice of any Chinese art and is at the root of Chinese medical theory. According to Chinese thought, Qi is an invisible energy-like phenomenon which is present in every animate or inanimate object in the universe. It is a difficult concept to explain but Qi can almost be thought of as an adhesive which holds the cosmos together; the inertia through which all is create and destroyed. The ancient Chinese philosophy holds that Qi is this most basic substance constituting the world. Accordingly, TCM also believes that Qi is the most fundamental substance in the construction

of the human body and in the maintenance of its life activities. Therefore, any substantial matter can be regarded as a special process of the movement of Qi, and life, in essence, is the course of Qi's ascending, descending, exiting and entering movements in given conditions.

3.5. The relations between Qi and blood, Yin and Yang

In TCM theory, blood and Qi are inseparable. Blood is the "mother" of Qi; it carries Qi and also provides nutrients for its movement. In turn, Qi is the "commander" of the blood. This means that Qi is the force that makes blood flow throughout the body and provides the intelligence that guides it to the places where it needs to be. Losing too much blood causes an overall Qi deficiency. When there is a Qi deficiency, the body cannot function properly and therefore presents with a fever. In the treatment of such Blood Deficiency, supplementing Qi plays an even more important role than nourishing Blood. Bleeding, for another example, may be the result of Qi deficiency because Qi controls Blood flow, so such bleeding should be treated by strengthening Qi. TCM understands that everything is composed of two complementary energies; one energy is Yin and the other is Yang. They are never separate; one cannot exist without the other. Yin and Yang come from Qi. Qi is required to harmonize Yin and Yang.

4. Mitochondrial energy metabolism – Its related diseases and ageing

After the symbiotic engulfment of aerobic α -proteobacteria by pre-eukaryotic cells more than 1.5 billion years ago, mitochondria evolved as specialized organelles with a plethora of cellular functions. Over recent years, mitochondria have taken center stage as remarkably autonomous and dynamic cellular organelles that are intimately involved in orchestrating a diverse range of cellular activities. Mitochondria regulate the life and death of cells by manipulating several factors, including bioenergetics, mitochondrial permeability transition, and mitochondrial redox-status, they are usually regarded as specialized organelles for cellular respiration and oxidative phosphorylation (OXPHOS). Mitochondria are the driving force behind life, over 80% of the energy which is required by an adult is produced by OXPHOS under normal physiological condition. Energy metabolism would be regulated by the relative amount of adenosine triphosphate (ATP) available, as described by adenylate energy charge (AEC). ATP has been called the energy "currency" of the cell. The electron transport chain (ETC) in the mitochondrial inner membrane is actively involved in ATP synthesis in combination with respiration. The impaired ETC works less efficiently in ATP synthesis and generates more reactive oxygen species (ROS), which will cause further oxidative damage to various biomolecules. In the aging process, oxidative damage ultimately leads to a progressive decline in bioenergetic function and enhanced mitochondrial oxidative stress. Lower ATP levels can decrease the efficiency of energy-dependent processes and ATP-mediated signal transductions. Inadequate ATP availability would initiate and accentuate the adverse consequences of energy-dependent pathways. The energy depletion and enhanced oxidative stress can lead to the aging process. As the "hubs" for cellular metabolism, mitochondria are crucial for both life and death of eukaryotic cells, and are the main switch of cell apoptosis.

Dysfunction of mitochondria has severe cellular consequences and is linked to ageing and neurodegeneration in human. Since discovery of the first case of mitochondrial disease in 1959, with the depth of mitochondrial research and the rapid development of mitochondrial medicine, the number of mitochondria-related diseases is rapidly amplified, mitochondrial dysfunction would undermine the function of cells, tissues and organs, thereby causing cancer, myasthenia gravis, obesity, stroke, cardiovascular disease (ischemic reperfusion injury, hypertension, coronary heart disease, heart failure, diabetes and atherosclerosis, etc.), age-related diseases, neurodegenerative diseases (Parkinson's disease, Alzheimer's disease, depression etc.), and aging etc. These diseases is today's major diseases that threaten human health, mitochondria has become a new target for the treatment of diseases, because mitochondrial oxidative damage is the main reason for cell damage and death, the general treatment program to treat a variety of mitochondrial diseases is the reduction in mitochondrial oxidative damage. Therefore, mitochondrial protection is an important mechanism for the treatment of mitochondrial-related diseases.

Bioenergetics research in life sciences have played an important role, Mitchell's chemiosmotic theory earned the 1978 Nobel Prize in Chemistry, as the coupling between electron transport in the respiratory chain and adenosine diphosphate (ADP) phosphorylation which is caused by electrochemical gradient of protons between inner and outer mitochondrial membrane was expounded; Nobel Prize in Chemistry in 1997 was awarded academician PD Boyer in the U.S. Academy of Sciences for elucidating generation mechanism of ATP—the most important energy molecules. The work was closely related to the energy production and consumption which is required for life activities. According to the modern life science, energy metabolism is the center for life activity, if the energy metabolism is normal, the body can carry out normal vital activities, if no bio-energy is supplied for the body, the life activities cease immediately. Therefore, Qi and bioenergy have identical functions.

4.1. Energy metabolism in mammalian cells

Mitochondria have been described by cytologists since the mid 19th century. According to Scheffler [4], the term mitochondrion was coined by Benda in 1898. However, only in the mid 20th century the role of the mitochondria in oxidative energy metabolism was established in detail [5]. All cells in the body depend on a continuous supply of ATP in order to perform their different physiological and biochemical activities. Mitochondria have a central role in the energy metabolism. Part of the free energy derived from the oxidation of food inside mitochondria is transformed to ATP, energy currency of the cell. This process depends on oxygen. When oxygen is limited, glycolytic products are metabolized directly in the cytosol by the less efficient anaerobic respiration that is independent of mitochondria. The following describes the basic processes occurring in a typical normal cell, using glucose as a major source of energy (Figure 1). The breakdown of glucose into water and CO₂ includes two steps, namely, glycolysis (the anaerobic phase) taking place in the cytoplasm, and OXPHOS (the aerobic phase) occurring in the mitochondria. Of the total yield of 38 ATP per mole of glucose, two are produced in the glycolysis process and 36 during the OXPHOS. It is important to note that oxygen availability in the mitochond-

tion is a critical factor for the normal ATP production in the cell. The end product of glycolysis, pyruvate, is transported into the mitochondria by a specific carrier protein. The pyruvate is transformed, in the matrix of the mitochondria, into acetyl coenzyme A that activates the tricarboxylic acid (TCA) (Krebs) cycle. In the absence of oxygen, the end product of pyruvate is lactate that may leave the cell and pass into the microcirculatory blood stream via the monocarboxylase transporter located in the plasma membrane [6].

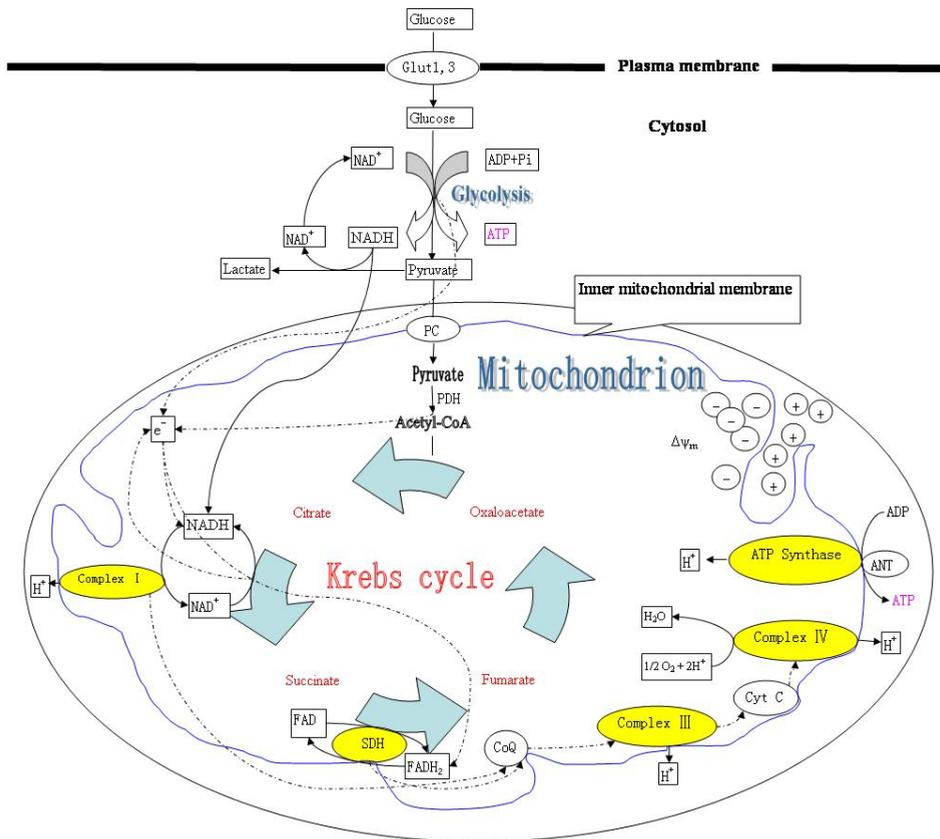


Figure 1. Overview of the cellular energy metabolic pathways. Mitochondria can metabolize fuels, such as fatty acids, amino acids and pyruvate, derived from glucose. When glucose enters the cell via glucose transporters, it is metabolized by glycolysis to pyruvate. Pyruvate prevalently enters mitochondria through its specific carrier (PC), with only a small amount being metabolized to lactate due to the excess of NADH. In mitochondria, pyruvate dehydrogenase complex (PDH) converts pyruvate into acetyl-CoA, which feeds into the Krebs cycle, of which the net reactive result is to generate NADH and FADH₂. The respiratory chain consists of four enzyme complexes (complexes I-IV) (yellow), and two mobile carriers (coenzyme Q and cytochrome c) along which the electrons liberated by the oxidation of NADH and FADH₂ are passed, and ultimately transferred to oxygen. This respiratory process which electrons pass through generates membrane potential ($\Delta\psi_m$)—the main driving force for ATP synthesis used by the ATP synthase to phosphorylate ADP and produce ATP, that in turn is carried to the cytosol by adenine nucleotide translocase (ANT) in exchange for ADP.

The mitochondrial ATP production relies on the ETC, composed of respiratory chain complexes I–IV, which transfer electrons in a stepwise fashion until they finally reduce oxygen to form water. The NADH and FADH₂ formed in glycolysis, fatty-acid oxidation and the citric acid cycle are energy-rich molecules that donate electrons to the ETC. Electrons move toward compounds with more positive oxidative potentials and the incremental release of energy during the electron transfer is used to pump protons (H⁺) into the intermembrane space. Complexes I, III and IV function as H⁺ pumps that are driven by the free energy of coupled oxidation reactions. During the electron transfer, protons are always pumped from the mitochondrial matrix to the intermembrane space, resulting in a potential of ~150–180 mV. Proton gradient generates a chemiosmotic potential, also known as the proton motive force, which drives the ADP phosphorylation via the ATP synthase (F₀F₁ ATPase i.e., complex V). F₀ domain of ATPase couples a proton translocation across the inner mitochondrial membrane (IMM) with the phosphorylation of ADP to ATP [7]. The energy-transducing function is maintained by the mitochondrial inner membrane and over 95% of total cellular ATP is supplied by mitochondrial phosphorylation [8]. Cellular activities can, therefore, be adversely affected by damage to the mitochondrial energy-transducing functions [9]. The rate of mitochondrial respiration depends on the phosphorylation potential expressed as a [ATP]/[ADP] [Pi] ratio across the IMM that is regulated by the adenine nucleotide translocase (ANT). In the case of increased cellular energy demand when the phosphorylation potential is decreased and more ADP is available, a respiration rate is increased leading to an increased ATP synthesis. There is usually a tight coupling between the electron transport and the ATP synthesis and an inhibition of ATP synthase will therefore also inhibit the electron transport and cellular respiration. Under certain conditions, protons can reenter into mitochondrial matrix without contributing to the ATP synthesis and the energy of proton electrochemical gradient will be released as heat. This process, known as proton leak or mitochondrial uncoupling, could be mediated by protonophores (such as FCCP) and uncoupling proteins (UCPs) [10]. As a consequence, uncoupling leads to a low ATP production concomitant with high levels of electron transfer and high cellular respiration [11].

From its role as the cellular powerhouse, the mitochondrion is emerging as a key participant in cell death. Apoptosis and necrosis are two alternative forms of cell death, with well-defined morphological and biochemical differences [12]. One crucial physiological difference between cells that undergo apoptosis or necrosis is intracellular ATP level. Complex I plays a major role in mitochondrial OXPHOS, include oxidizing NADH in the mitochondrial matrix, reducing ubiquinone to ubiquinol and pumping protons across the inner membrane to drive ATP synthesis [13]. Since its inhibition results in incomplete mitochondrial electron transport and disturbance of mitochondrial energy metabolism, dysfunction of Complex I in the hippocampus during the initial prolonged epileptic seizure may conceivably lead to necrosis because of a decrease in ATP production [14]. Mitochondrial creatine kinase is an important component of the cellular energy buffering and transport system, connecting oxidative phosphorylation to ATP consumption. The reduced activity of creatine kinase may lead to a decreased cellular ATP/ADP ratio [15].

4.2. The dysfunction of mitochondrial energy metabolism and human diseases

The role of mitochondria in mammalian cells is generally presented as a "central pathway" for energy metabolism, but mitochondria house many additional metabolic pathways and play a key role in apoptosis, free radical production, thermogenesis and calcium signaling. As a consequence, impairment of mitochondrial function is associated with a clinically heterogeneous group of human disorders, often referred to as mitochondrial cytopathies [16]. In recent years, much attention has been attributed to the dysfunction of mitochondrial energy metabolism, which has not only been associated with cardiac failure but also to numerous other disorders, such as cancer, diabetes, obesity and general senescence. The mitochondrion hosts the enzymes of the Krebs cycle and the complexes of the ETC which generate ATP by oxidation of carbohydrates, fatty acids and amino acids. It therefore functions as the foremost supplier of energy substrate to maintain systemic energy balance and homeostasis [17]. Mitochondrial OXPHOS serves a central role for energy homeostasis in mammals. Impaired mitochondrial OXPHOS contributes to the pathogenesis of a wide range of disease conditions, including metabolic disorders, neurodegeneration, and heart failure. Genetic control of mitochondrial biogenesis and function has been an active area of research in recent years [18].

In addition to the mitochondrial role in cellular bioenergetics, the pivotal role of mitochondrial dysfunction in various human diseases has become increasingly clear. For example, the involvement of the mitochondria in tumor cell pathogenesis was initially described by Warburg 80 years ago, and later followed by many studies. The pioneering work of Warburg on the metabolism of tumors led to the hypothesis that the development of cancer may originate when cellular glycolysis increases, while mitochondrial respiration becomes impaired [19-21]. Warburg's hypothesis, termed the "Warburg effect", explains the significance of cellular energy metabolism in the pathophysiology of cancer cells. Since then, a large body of investigations has shown the involvement of the mitochondria in many human diseases.

Mitochondrial oxidative damage is a major factor in many human disorders, including mitochondrial hepatopathies, chronic hepatitis C, steatosis, early graft dysfunction after liver transplantation, ischemia-reperfusion injury, ageing and inflammatory damage [22]. Oxidative damage accumulates more in mitochondria than in the rest of the cells because electrons continually leak from the respiratory chain to form damaging ROS. This oxidative damage may modify mitochondrial proteins, DNA and lipids which may lead to mitochondrial bioenergetics failure leading to necrotic or apoptotic cell death [23]. Despite the collection of vast knowledge on the mitochondrial function and human health, the accumulated information did not translate into practical clinical tools, such as new drugs or medical devices.

Decreased levels of ATP and phosphocreatine were observed in brains of portacaval-shunted rats infused with ammonia [24] as well as in rats with chronic hepatic encephalopathy (HE) [25]. Reduced brain ATP levels were likewise reported in rats with acute hyperammonemia [26]. Further, decreased levels of ATP were observed in cultured astrocytes treated with ammonium chloride [27]. Recent studies have also indicated reduced levels of AMP and ADP in rats with acute hyperammonemia, and such decrease was found to be due to increased activity of AMP deaminase and adenosine deaminase [28,29]. Another possible mechanism for impaired energy metabolism in HE and hyperammonemia is the mitochondrial permea-

bility transition (MPT). The MPT is characterized by a sudden increase in the permeability of the IMM to small solutes (ions and other molecules <1500 Da). The MPT is due to the opening of the permeability transition pore (PTP) in the IMM, usually in response to an increase in mitochondrial Ca^{2+} levels. This leads to a collapse of the mitochondrial inner membrane potential that is created by the pumping out of protons by the electron transport chain. Loss of the membrane potential leads to colloid osmotic swelling of the mitochondrial matrix, movement of metabolites across the inner membrane (e.g., Ca^{2+} , Mg^{2+} , glutathione, and NADPH), defective OXPHOS, cessation of ATP synthesis, and the generation of ROS. The latter acts to further aggravate the MPT [30,31]. Ca^{2+} is a well known inducer of the MPT [32]. Mitochondrial ATP-sensitive K^+ channel (mito K_{ATP}) opening results in decreased mitochondrial ROS production. In addition, under energy deprivation conditions, mito K_{ATP} opening inhibits mitochondrial ATP hydrolysis by ATP synthase, which helps to keep the cytosolic ATP/ADP ratio and also to limit mitochondrial Ca^{2+} uptake, indirectly preventing MPT [15].

As noted in the above sections dealing with glycolysis, TCA cycle and OXPHOS, various animal models of acute liver failure (ALF) have been used to examine bioenergetic events in ALF. These studies described several abnormalities in cerebral energy metabolism, including glucose utilization [33], reduction in TCA cycle enzyme activity [34], decreased rate of respiratory chain activity [35], inhibition of creatine kinase activity [36], and reduced levels of ATP [37]. Studies showing the induction of the MPT in ammonia-treated cultured astrocytes, as well as in brains of rats with ALF suggest that the MPT plays a crucial role in the bioenergetic failure associated with HE and hyperammonemia. Hypertriglyceridemic liver mitochondria have a higher resting respiration rate but normal OXPHOS efficiency. The mild uncoupling mediated by mito K_{ATP} accelerates respiration rates and reduces ROS generation [38]. Since the mitochondria are involved in a wide range of diseases, a new therapeutic approach was developed 30 years ago, aimed to develop drugs targeting the mitochondria.

4.3. Mitochondrial energy metabolism and ageing

Ageing is a process characterized by a general decline in physiological functions, and it is also considered as a major risk factor for many age-related diseases, including, but not limited to, neurodegenerative diseases, cardiovascular disorders, and metabolic diseases [39-41]. Ageing can be defined as "a progressive, generalized impairment of function, resulting in an increased vulnerability to environmental challenge and a growing risk of disease and death". Ageing is likely a multifactorial process caused by accumulated damage to a variety of cellular components. During the last 20 years, gerontological studies have revealed different molecular pathways involved in the ageing process and pointed out mitochondria as one of the key regulators of longevity. Increasing age in mammals correlates with increased levels of mitochondrial DNA (mtDNA) mutations and a deteriorating respiratory chain function. Experimental evidence in the mouse has linked increased levels of somatic mtDNA mutations to a variety of ageing phenotypes, such as osteoporosis, hair loss, graying of the hair, weight reduction and decreased fertility. A mosaic respiratory chain deficiency in a subset of cells in various tissues, such as heart, skeletal muscle, colonic crypts and neurons, is typically found in aged humans. It has been known for a

long time that respiratory chain-deficient cells are more prone to undergo apoptosis and an increased cell loss is therefore likely of importance in the age-associated mitochondrial dysfunction [42]. In this part, I'd like to point out the link between the mitochondrial energy balance and ageing, as well as a possible connection between the mitochondrial metabolism and molecular pathways important for the lifespan extension.

Mitochondrial theory of ageing: Even though the process of oxidative phosphorylation is efficient, a small percentage of electrons may "leak" from the ETC, particularly from complexes I and III, during normal respiration and prematurely reduce oxygen, forming ROS [43]. Mitochondria is a well known source of cellular ROS; when an electron escapes from the mitochondrial electron transport chain, especially at complex I or III, it may react with molecular oxygen to form superoxide ion. Superoxide ion constantly generated during cellular metabolism gets converted to hydrogen peroxide (H_2O_2) and other ROS. Under physiological conditions, the maintenance of an appropriate level of intracellular ROS is important in keeping redox balance and signaling cellular proliferation [44]. ROS produced within mitochondria presents almost 90% of the total ROS produced in the cell. The fact that the mitochondrial ETC is the major ROS production site leads to the suggestion that mitochondria are a prime target for oxidative damage and hence the mitochondrial theory of ageing, a correlate to the free radical theory [45]. Over the years, substantial evidence has emerged from morphological, bioenergetic, biochemical and genetic studies to lend support to this theory [42].

Despite conflicting views concerning the primary role of mitochondrial ROS as a cause of aging [46], the generation of ROS within mitochondria remains the most viable theory to explain the process of aging. Increased levels of ROS within mitochondria are the principal trigger not only for mitochondrial dysfunction, but also for diseases associated with aging in general [47]. The latest results strongly argue that the observed phenotypes in mtDNA mutator mice are a direct consequence of the accumulation of mtDNA point mutations in protein-coding genes, leading to a decreased assembly of mitochondrial ETC complexes, respiratory chain dysfunction and thus to premature ageing [48].

On the other hand, the "uncoupling to survive" theory proposes that energy metabolism is in a positive relation with longevity. This theory is also based on the notion that inefficiency in the mitochondrial ATP generation may be necessary to reduce ROS generation in the cell [49]. High proton motive force that drives an efficient ATP synthesis comes with an additional cost, the production of ROS. Because ROS production is highly dependent on the proton motive force, proton leak might help to limit the oxidative damage. There are a number of articles suggesting that UCPs could play an important role in this process. It has been proposed that UCPs have a role in the protection from oxidative damage by lowering a proton motive force thus causing a "mild" uncoupling and the attenuation of superoxide production from electron chain [49]. During "mild" uncoupling, caused by UCPs activation with superoxide and other ROS products derived upon oxidation of membrane phospholipids, ATP is still synthesized, a respiration rate is increased and in parallel a ROS production is decreased [49,50].

A significant decrease in the mitochondrial bioenergetic capacity with advancing age has been shown in numerous animal models and recently in a study of human volunteers [51]. A study on aged rats showed an increased intra-mitochondrial ROS production and oxidative damage,

increased proton leak rates resulting in a depletion of membrane potential and a reduction of ATPase and complex IV activities. Treatment of aged rats with the insulin-like growth factor 1 (IGF1) corrected these parameters indicating that a cytoprotective effect of IGF1 is closely related to the mitochondrial protection [52]. Caloric restriction is the only dietary intervention that consistently increases median and maximal lifespan in organisms ranging from yeast to mammals. This dietary regime implies 20–50% restriction of the overall caloric intake of animals on *ad libitum* regime [53]. The precise molecular mechanisms of the life-extending actions of caloric restriction still remains unclear, but most likely mitochondrial energy metabolism plays a very important role in this process.

The age-related increase in mitochondrial oxidative stress can disrupt mitochondrial structural and functional integrity, thereby triggering a vicious cycle of ROS generation. Experimental findings indicate that the age-related decrease in mitochondrial respiratory efficiency was associated with the significant decline in respiratory complex (I-V) activities, presumably mediated by self-inflicted oxidative damage [54]. In addition, the extent of oxidative damage on key metabolic enzymes increases with age, with consequent decreases in substrate binding affinity and mitochondrial ATP generation capacity [55]. The oxidation of DNA, RNA, protein and lipid molecules in mitochondria and other cellular components can culminate in functional impairment in cells, tissues, and ultimately in vital organs such as brain, heart and liver [56]. Taken together, the capacity to produce ATP and respond to cellular stress decrease as a function of age during the age-associated deterioration of mitochondrial structure and function. The mitochondrial dysfunction results in increased ROS generation, which tilts the cellular environment towards an oxidative state (i.e., impairment of cellular redox balance) and increases the susceptibility to diseases associated with aging [57].

Studies that link mitochondrial respiration/ATP production and longevity are needed to clarify the role of mitochondrial biogenesis, mitochondrial respiration rate and ROS production in different aspects of ageing. However, mitochondria are today in the scientific spotlight and sure hold promises for the future ageing research. That is certainly enough to make mitochondria a center of our attention.

5. Qi-invigoration and Yang-invigoration

According to TCM theory, in order to have good health you must have sufficient Qi and your internal organs must work in harmony with each other, as long as sufficient Qi flows freely through the meridians and your organs work in harmony your body can remain healthy. If there isn't enough Qi, one or more organs can become imbalanced and develop energy function disorders. TCM frequently references several major Qi states of imbalance. One is an overall "Qi deficiency", which is often described in Western medical terms as chronic fatigue syndrome (CFS), may affect the Lungs with symptoms of shortness of breath, the Stomach/Spleen with symptoms such as poor appetite and the body in general with symptoms of fatigue and weakness. Most treated CFS by invigorating Qi and Yang. For an explanation of TCM, the ultimate reasons for the symptoms described earlier are induced by deficiencies in five organs

(including Qi, Blood, Yin and Yang deficiencies) caused by the invasion of an exogenous pathogen, excessive physical strain (manual labor, mental labor and sexual intercourse), abnormal emotional states (elation, anger, worry, anxiety, sorrow, fear and terror) or an improper diet.

Chinese tonic herbs that can produce health-promoting action are used for the treatment of various patterns of deficiency in body function with respect to Qi, Blood, Yin, or Yang, and their combinations. These types of functional imbalance are viewed as sub-healthy conditions in modern medicine. Chinese tonic herbs are generally classified into four categories on the basis of their health-promoting actions, namely, "Qi-invigorating", "Blood-enriching", "Yin-nourishing" and "Yang-invigorating". Of these four types of tonic herbs, the "Qi-invigorating" and "Blood-enriching" herbs are grouped under the "Yang" family and "Yin" family, respectively. Maintaining Yang and Yin in harmony is akin to attaining the homeostatic state in modern medicine [58]. Yang Qi refers to the body's vital force or functional aspects in general. Unlike the Blood or Body Fluids, Qi is an abstract concept in TCM; it can't be seen and belongs to Yang. Yang Qi sometimes refers to some body qualities and functions like superficial, upward direction, hyper-functioning, stimulating and light. It is the opposite of Yin Qi. Yang deficiency indicates insufficiency of Yang Qi inside the body that fails to provide the functions of warmth, motivation and promotion. Symptoms or signs include aversion to cold, cold limbs, bland taste in the mouth, preference for hot drinks, pale complexion, spontaneous sweating, general swelling, profuse and clear urine or loose stool. The tongue is pale, bulky with a white slimy coating, and the pulse is deep and slow or thready on examination.

According to TCM theory, Yang is viewed as a manifestation of body function supported by various organs. A "Yang-invigorating" action therefore involves the general up-regulation of cellular activities. As ATP, an energy-rich biomolecule, is universally used for energizing cellular activities, the "Yang-invigorating" action may be mediated by the enhancement of mitochondrial ATP generation [59]. "Yang-invigorating" Chinese tonic herbs have shown to enhance the myocardial mitochondrial ATP generation capacity in mice *ex vivo* and in H9c2 cardiomyocytes [58,60]. All "Yang-invigorating" Chinese tonic herbs dose-dependently enhanced the mitochondrial ATP generation capacity. The stimulation of ATP generation was associated with an increased extent of mitochondrial electron transport [60]. It is believed that the up-regulation of cellular activities by "Yang-invigoration" in Chinese medicine requires an increased supply of ATP, which is in turn largely supported by mitochondrial OXPHOS [58].

Holistically, it is believed that the Yang-invigorating herbs enhance physiological cellular activities, which is in turn critically dependent on mitochondrial ATP generation through the OXPHOS process at the cellular level. A previous study has shown that short-term oral treatment with the methanol extract of Yang-invigorating herbs, including Cortex Eucommiae, Herba Cistanches, Herba Cynomorii, Rhizoma Curculiginis, Herba Epimedii, Radix Dipsaci, Rhizoma Drynariae, Fructus Psoraleae, Semen Cuscutae, Radix Morindae, and Semen Allii, enhanced myocardial ATP generation and produced significant stimulatory action on pyruvate-supported mitochondrial electron transport in mice [60]. This finding is corroborated by a recent study involving Yang and Yin tonic herbs using a cell-based assay of ATP-generating capacity, which showed that Yang but not Yin tonic herbs enhanced mitochondrial

ATP generation capacity in H9c2 cardiomyocytes [58]. Moreover, long-term treatment with a Yang-invigorating Chinese herbal formula (VI-28; composed of Radix Ginseng, Cornu Cervi, Cordyceps, Radix Salviae, Semen Allii, Fructus Cnidii, Fructus Evodiae and Rhizoma Kaempferiae) was found to enhance mitochondrial ATP generation in brain, heart, liver and skeletal muscle tissues of male and female rats [61].

Emerging evidence has suggested that in addition to up-regulating mitochondrial functional status, Yang tonic herbs also enhance cellular/mitochondrial antioxidant capacity, and may thus prevent age-related diseases and prolong the healthspan. The proposed biochemical mechanism underlying the antioxidant action of Yang tonic herbs involves a sustained and low level of mitochondrial ROS production, which is secondary to the increased activity of the ETC, with the possible involvement of mitochondrial uncoupling. "Yang invigoration" improves antioxidant defense in the body in the long term and thereby offers a promising prospect for preventing or possibly delaying age-related diseases and the detrimental effects of aging [62]. Studies from various laboratories showed that Yang tonic herbs produced antioxidant actions by free radical-scavenging, inhibition of oxidant production, inhibition of NADPH-dependent lipid peroxidation and increase of antioxidant enzyme activities, with a resultant protection against oxidative tissue damage. These findings were consistent with the earlier study which showed that Yang tonic herbs possessed stronger free radical scavenging activity than that of tonic herbs of other functional categories [63].

A growing body of evidence has revealed the crucial involvement of mitochondrial dysfunction and impaired antioxidant status in the pathogenesis of various age-related diseases and the aging process in general [64,65]. Yang tonic herbs/ formulae, which can induce endogenous mitochondrial antioxidant status and functional capacity enhancement [66], may therefore offer a promising prospect for preventing or possibly delaying age-related diseases and the detrimental effects of aging. With respect to Chinese medicine, more than 50% of the elderly people in China were found to show a deficiency of Yang (or Qi) in body function [67], and Yang (or Qi) tonic herbs/formulae are therefore commonly used for retarding the adverse consequences of aging in the practice of Chinese medicine. According to TCM theory, a deficiency of Yang is believed to be one of the causative factors for the development of Parkinson's disease (PD), a common neurodegenerative disease that severely compromises the quality of life in many elderly individuals [68]. Based on the finding that ViNeuro can enhance the mitochondrial ATP generation capacity (a "Yang-invigoration" property), it is plausible that the relief of Parkinsonian symptoms involves an improvement of cellular energy status that eventually leads to an enhancement of neuronal function [62].

TCM frequently references several major Qi, or energy function, problems. One is an overall "Qi deficiency". Qi deficiency is the common cause of a variety of diseases and Qi-invigoration is the basic principle for treatment of Qi deficiency. Doctors of TCM usually compose prescriptions made up of Qi-invigorating herbal medicines (QIHM) for Qi deficiency, and have accumulated abundant clinical experience for a long time. QIHM is a kind of herbal medicines which can invigorate Qi and treat syndromes of Qi deficiency, they have the effects of invigorating Qi, promoting the production of body fluid and tonifying the Spleen and Lung etc. Within the body, Qi is present in all active aspects of the body, so is considered to be a

Yang substance. Due to the popularity and therapeutic values of "Qi-invigorating" herbs, the investigation of biological activities and the underlying mechanisms in relation to "Qi-invigoration" is of great pharmacological interest. In this regard, our earlier study has demonstrated the relationship between "Qi-invigorating" action and bioenergetic level in skeletal muscle of "Qi-invigorating" herb-treated rats [69]. However, the pharmacological basis of "Qi-invigorating" action has yet to be established. To investigate the mechanism of Qi-invigoration in TCM, the following experiment was performed.

6. Research ideas on Qi-invigoration – Mitochondrial energy metabolism perspective

Sijunzi Decoction (SD), a Chinese recipe issued firstly in the ancient pharmacopeia of the Song Dynasty, "*Taiping Huimin Heji Jufang*", having effects of reinforcing the asthenia Qi, is one of the classic recipes. The recipe consists of ginseng root, white atractylodes rhizome, Poria cocos and honey-fried licorice root. As the traditional Qi-invigorating and spleen-tonifying prescription, SD experienced repeated clinical validation by the many TCM practitioners for hundreds of years, its prescription is concise, compatibility is decent, the effect is exact, and it is highly regarded. A number of Qi-invigorating prescriptions are derived based on it, and it is a basic prescription for Spleen Qi deficiency syndrome, the series of Qi-invigorating prescriptions derived from SD are widely used for clinical treatment of many diseases, not only for digestive diseases, but also for the treatment of chronic hepatitis, chronic nephritis, and coronary heart disease etc. Pharmacological studies show that SD has anti-aging, anti-fatigue, anti-hypoxia, antioxidant and immune-improving function. SD can enhance mitochondrial succinate dehydrogenase, cytochrome oxidase activity and relieve the mitochondrial injury of the Spleen Qi deficiency rats. In such case, SD was selected for investigating Qi-invigorating role to make it more representative.

Our previous studies show that all the four Qi-invigorating herbal medicines (QIHM) (including ginseng, astragalus root, pilose asiabell root, white atractylodes rhizome) can increase levels of ATP, adenylate energy charge (AEC), total adenylate pool (TAP); on the contrary, all the four Qi-flow regulating herbal medicines (QRHM, including immature bitter orange, magnolia bark, green tangerine and lindera root) can decrease levels of ATP, AEC and TAP in liver cells. In a word, QIHM and QRHM increase and decrease bioenergy level of liver cells respectively *in vivo*. Therefore, Qi is closely related to bioenergy [3]. Previous experimental findings have demonstrated that all "Yang-invigorating" herbs are capable of enhancing mitochondrial ATP generation capacity (ATP-GC) in both cell and animal studies [58,70]. As a subcategory of "Yang-invigorating" herbs, "Qi-invigorating" herbs may also stimulate mitochondrial ATP-GC in various tissues. In a study, using Renshen (*Panax ginseng*), Xiyangshen (*Panax quinquefolius*) and Dangshen (*Codonopsis pilosulae*), the effect of "Qi-invigorating" Chinese tonic herbs on mitochondrial ATP-GC using *in situ* and *ex vivo* assay systems were investigated. The results showed that the three tested "Qi-invigorating" Shens in Chinese medicine stimulated the ATP-GC *in situ* in the cell-based assay system [71]. Further investi-

gations should examine whether representative "Qi-invigorating" herbal formula SD can stimulate mitochondrial ATP-GC *in vivo*.

Although Qi of TCM is similar to the concept of modern medical bioenergy in some aspects, the mechanism of Qi-invigoration still lacks convincing evidence. Therefore, I take it as my basic point to approach the characteristics of SD on energy metabolism from the production (oxidative phosphorylation) and regulation (adenylate energy charge) of bioenergy (ATP). Since there is no direct detection method on Qi, the Qi-invigorating representative prescriptions SD were selected to study the effect on dysfunction of energy metabolism caused by Qi deficiency to investigate the mechanism of Qi-invigoration in TCM.

6.1. Materials and methods

6.1.1. Animals and materials

Male Kunming mice were purchased from Experimental Animal Center, Dalian University. Spherisorb C₁₈ reversed-phase chromatographic column (4.6 mm×250 mm, 5 μm particle size) was purchased from Dalian Institute of Chemistry and Physics, Chinese Academy of Sciences. Adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), L-glutamic acid, and DL-malate were from Sigma Chemical (St Louis, MO, USA). Ginseng root, white atractylodes rhizome, Poria cocos, honey-fried licorice root, immature bitter orange, magnolia bark and Rhizoma et Radix Rhei Palmat were purchased from Beijing Tongrentang Drugstore, and identified by professor Li Jiashi at Beijing University of Chinese Medicine. They are *Panax ginseng* C.A. Mey (Tongrentang red ginseng), *Atractylodes macrocephala* Koidz, *Poria cocos* (Schw.) Wolf, *Glycyrrhiza uralensis* Fisch., *Citrus aurantium* L., *Magnolia officinalis* Rehd et Wils, and *Rheum palmatum* L. respectively.

6.1.2. Preparation of Sijunzi Decoction (SD) and Xiaochengqi Decoction (XD)

SD and XD were prepared by hot-water extraction. Powdered dry Ginseng root, white atractylodes rhizome, Poria cocos and honey-fried licorice root (1:1:1:1) were immersed in distilled water (the ratio of the drug and distilled water was 1:10) for 2 hours and extracted thrice for 0.5 hour each in a boiling water bath. The filtrate was collected after filtration with gauze, mixed and condensed to 0.5 g crude drug/mL under a reduced pressure and then centrifuged at 3000 rpm for 10 min. The supernatant (SD) was collected and stored at 4°C. XD [Rhizoma et Radix Rhei Palmat, magnolia bark and immature bitter orange (4:5:3)] was prepared by the same way as SD and condensed to 2.5 g crude drug/mL.

6.1.3. Spleen Qi deficiency model

Spleen Qi deficiency model was established by exhaustion, dissipating stagnant Qi and irregular diet which was induced by XD and semi-starvation. Forty mice were randomly divided into four groups: Normal group, model group, SD low dose group (SDL) and SD high dose group (SDH). Normal group mouse was administered normal saline (10 mL/kg/day) for 43 days by oral gavage. All the other group mouse was administered an oral dose of XD (60

g/kg/day) for 15 days and was fed with half-full diet once every other day, then the model group mouse was killed for analysis. SDL and SDH mice were given an oral dose of SD (8 and 16 g/kg/day respectively) for 28 days and were killed on forty-fourth day for detection. All the mice were maintained with free access to drinking water.

6.1.4. Isolation of liver mitochondria

Mitochondria were isolated by differential centrifugation using a modified protocol of Fink et al. [72]. Protein determinations were carried out by Bradford method using BSA as a standard [73].

6.1.5. Measurement of ATP, ADP, and AMP in skeletal muscle cells by HPLC

Briefly, determination of ATP, ADP, and AMP in cells of skeletal muscle from the thigh of mice, which was carried out with our previous method [74], by gradient RP-HPLC (reversed-phase high performance liquid chromatography) with ultraviolet detector at room temperature. ATP, ADP and AMP contents in liver cells was calculated by computing the peak area of standard solutions of nucleotides with known concentrations. Total adenylate pool (TAP) and adenylate energy charge (AEC) were calculated by the following formulas respectively: $TAP = [ATP] + [ADP] + [AMP]$, $AEC = ([ATP] + 0.5[ADP])/TAP$.

6.1.6. Measurement of liver mitochondrial respiratory function

Respiratory function of liver mitochondria was measured using the Clark-type oxygen electrode method described by Estabrook [75] with slight modifications [3]. Respiratory state 3 and 4 can be calculated according to the OXPHOS curve. Respiration rates were expressed in nanomoles atom O per minute per milligram of protein. Respiratory control ratio (RCR) was the ratio of state 3 to state 4 respiration. P/O ratio is the number of ADP molecules phosphorylated per oxygen atom reduced.

6.1.7. Statistical analysis

Data were expressed as means \pm SD and statistical differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by least significant difference (LSD) *post hoc* multiple comparisons test using the statistical software package SPSS 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Results were considered statistically significant at the probability (*P*) values < 0.05 level.

6.2. Results and discussion

6.2.1. Effects of SD on energy state in skeletal muscle cells of mice under Qi deficiency *in vivo*

The Spleen and the Stomach—especially the Spleen—are in charge of producing Qi and blood to nourish the body, particularly the muscles. In Chinese medicine, the Spleen is related to the muscles. Qi is closely related to bioenergy according to the ancient concept of Qi and modern bioenergetics [3]. Therefore, skeletal muscle was used for investigating energy level change of Qi deficiency mice. Impaired mitochondrial ATP formation may be the key

characteristic of Qi deficiency. I found that Qi deficiency led to a marked fall in cellular ATP, and a rise in cellular AMP associated with decreases in ATP/ADP and ATP/AMP ratios. The changes in ATP/ADP ratio might significantly influence mitochondrial membrane potential ($\Delta\psi_m$) [76]. The cellular AMP/ATP ratio was monitored as an index of metabolic stress [77]. Through the action of adenylate kinase (AK), any decrease in the cellular ATP/ADP ratio is converted into a decrease in the ATP/AMP ratio [78]. Qi deficiency elicits a marked decrease in the ATP/AMP ratio. The ATP/AMP ratio reduced from 27.4 of normal group to 6.25 under Qi deficiency conditions, whereas the ATP/ADP ratio reduced from 4.95 to 3.38. Qi deficiency has altered cellular energy state.

Adenylate energy charge (AEC) represents a linear measure of the metabolic energy stored in the adenine nucleotide system. Energy metabolism would be regulated by the relative amount of ATP available, as described by AEC. ATP has been called the "energy currency" of the cell, TAP is a measure of the cell energy status. TAP levels and AEC in muscle cells of model group were decreased compared with normal group. The AMP level in model group remained twofold higher than in normal group. SD treatment could increase ATP, TAP levels and ATP/ADP, ATP/AMP ratio, AEC in muscle cells in a dose-dependent manner. ATP/AMP ratio in SDH (16 g/kg/day) group increased over fivefold than in model group (Table 1).

Group	Dose (g/kg/day)	ATP (mM)	ADP (mM)	AMP (mM)	TAP (mM)	AEC	ATP/ADP	ATP/AMP
Model	-	1.01±0.21	0.29±0.13	0.16±0.08	1.46±0.29	0.78±0.07	3.38±0.84	6.25±4.3
Normal	-	1.66±0.31 ^c	0.34±0.11	0.06±0.04 ^b	2.06±0.38 ^b	0.89±0.08 ^b	4.95±0.69 ^c	27.4±7.8 ^c
SDL	8	1.35±0.23 ^b	0.32±0.12	0.09±0.06 ^a	1.76±0.34 ^a	0.85±0.05 ^a	4.26±0.74 ^a	14.8±5.7 ^b
SDH	16	1.68±0.28 ^c	0.35±0.15	0.05±0.05 ^b	2.08±0.40 ^b	0.90±0.07 ^b	4.82±0.85 ^b	33.8±8.3 ^c

All values are mean±SD (n=10). ^aP<0.05, ^bP<0.01, ^cP < 0.001 compared to model group.

Each value expressed in mM (ATP, ADP, AMP, TAP) or as a ratio (AEC, ATP/ADP, ATP/AMP).

SDL: SD low dose group; SDH: SD high dose group; ATP: adenosine triphosphate; ADP: adenosine diphosphate; AMP: adenosine monophosphate; TAP: total adenylate pool; AEC: adenylate energy charge.

Table 1. Effects of Sijunzi Decoction on adenylates level in skeletal muscle cells of mice *in vivo*.

Recently, a second mechanism of respiratory control has been found in eukaryotes. This control is based on the intramitochondrial ATP/ADP ratio, with a high ratio inhibiting oxidative phosphorylation through allosteric binding of ATP to a subunit of Complex IV. This inhibition is reversed when the concentration of ADP increases [79]. Energy metabolism would be regulated by AEC. In this study, we found that Qi deficiency significantly decreased AEC, which was reversed by SD accompanied by an increase in ATP. Thus, stimulation of ATP production by SD may be achieved through the regulation of the mitochondria by affecting the AEC response. This is consistent with the ability of *P. ginseng* in increasing ATP [75]. SD was able to enhance ATP production, cellular ATP levels are closely linked to mitochondrial function, which is regulated perhaps by AEC. SD was able to regulate AEC, possibly linked to mitochondrial ATP production. Data showed SD to be an enhancer of ATP production under

Qi deficiency induced anti-ATP circumstance. ATP levels were drastically lowered by Qi deficiency but SD stimulated an increased output of ATP.

The Spleen Qi-deficiency mice are characterized by lassitude of the limbs and poor appetite etc. In short, the TCM therapeutic approach of invigorating Qi and tonifying the spleen by SD can improve the mitochondrial energy metabolism of muscle cells as well as symptoms for the Spleen Qi-deficiency of experimental animals.

6.2.2. The effects of SD on liver mitochondrial respiratory function *in vivo*

Liver plays important role in metabolism to maintain energy level and structural stability of body. It is also site of biotransformation by which toxic compounds get transformed into less harmful products to reduce toxicity [80]. The state 3 respiration (oxygen consumption), the respiratory control ratio (RCR) values and P/O ratio of liver mitochondria of model mice driven by complex I substrates were all significantly decreased compared with the normal. Liver mitochondria isolated from SD treated rats showed significant decrease in state 3 respiration, RCR and P/O ratio, compared to the rates in mitochondria from models (Table 2). State 4 respiration was not significantly altered in SD treated rats. It showed that the efficiency of ATP production via ADP phosphorylation was decreased. Qi deficiency allows tissues to minimize their energy needs. In perfectly coupled mitochondria, there would be no proton leak across the IMM, and the entire gradient generated by the respiratory chain would be used to generate ATP [81]. Control of OXPHOS allows a cell to produce only the precise amount of ATP required to sustain its activities. Recall that under normal circumstances, electron transport and ATP synthesis are tightly coupled. The value of P/O ratio (the number of moles of Pi consumed for each oxygen atom reduced to H₂O) reflects the degree of coupling observed between electron transport and ATP synthesis [82]. Oxygen consumption increase dramatically when ADP is supplied. The control of aerobic respiration by ADP is referred to as respiratory control. Substrate oxidation accelerates only when an increase in the concentration of ADP signals that the ATP pool needs to be replenished. This regulation matches the rates of phosphorylation of ADP and of cellular oxidations via glycolysis, the citric acid cycle, and the electron transport chain to the requirement for ATP [79].

Group	Dose (g/kg/day)	State 3 (nmol/min/mg) ^d	State 4 (nmol/min/mg) ^d	RCR	P/O ratio
Model	-	66±10	18.8±2.5	3.5±0.5	2.08±0.33
Normal	-	82±12 ^b	19.4±1.8	4.2±0.6 ^a	2.59±0.28 ^b
SDL	8	60±14	18.7±2.3	3.2±0.7	1.90±0.24
SDH	16	56±11 ^a	18.3±2.2	2.9±0.6 ^a	1.78±0.22 ^a

^d nanomole O₂ per minute per milligram protein (nmol O₂ min⁻¹ mg protein⁻¹).

All values are mean±SD (n=10). ^aP < 0.05, ^bP < 0.01 compared to model group.

RCR: respiratory control ratio; SDL: SD low dose group; SDH: SD high dose group.

Table 2. Effects of SD on liver mitochondrial respiratory function *in vivo*.

Mitochondria produce significant amounts of cellular ROS via aberrant O_2 reaction during electron transport. This process in physiological conditions is tightly controlled with majority of ROS produced remaining inside intact mitochondria. The rate of mitochondrial respiration and ROS formation is largely influenced by the coupling state of the mitochondria [83]. SD decrease oxygen consuming rate and RCR of liver mitochondria maybe by improving of mitochondrial energy status (Figure 2), therefore, reduce mitochondrial ROS production. We consider this is appearance of lowering standard metabolic rate and is a kind of protective adaptation. Qi deficiency patients need nutritional supplements, adequate rest, and should reduce energy consumption, SD can just achieve this goal. It is conceivable that impairment of mitochondrial ATP production and the resulting energy depletion can lead to apoptosis. Aging-associated declines in mitochondrial respiratory function can lead to lower ATP production and higher oxidative stress. Lower ATP levels can decrease the efficiency of energy-dependent processes and ATP-mediated signal transductions [84]. An explanation of the protective effects of SD on mitochondria is based on the improvement of cellular energy status.

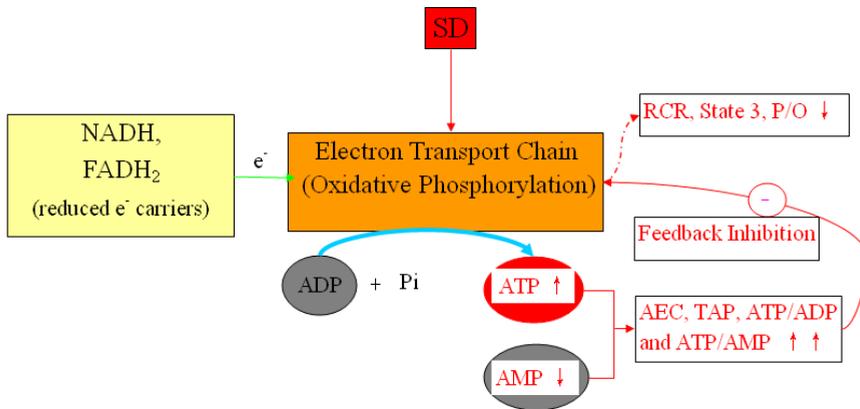


Figure 2. The action site of mitochondria as potential targets for SD therapy. SD treatment could increase ATP and TAP, decrease AMP levels and increase ATP/ADP, ATP/AMP ratio, AEC in muscle cells which feedback inhibit OXPHOS by decreasing RCR, state 3 respiration and P/O ratio of liver mitochondria. This is the result of improved mitochondrial energy metabolism and bioenergetic level and the potential Qi-invigoration mechanism of SD.

7. Conclusion

Qi is the hub from basic theory to clinical practice and health longevity in TCM. The true foundation of TCM is Qi. All kinds of diseases and ailments are born from Qi, Qi deficiency is the common cause of a variety of diseases and can lead to mitochondrial energy metabolism dysfunction, and Qi-invigoration is the basic principle for treatment of Qi de-

iciency. However, the mechanisms for Qi-invigoration in TCM remain elusive. We propose a hypothesis that Qi is closely related to bioenergy according to the ancient concept of Qi and modern bioenergetics [3], which is the entry point; all the QIHM have the regularity of same pharmacological effects, such as exercise capacity improvement, anti-fatigue, anti-oxidation and anti-apoptosis, etc. which are all closely related to mitochondrial function, which is the basis of study; Qi-invigorating prescriptions and QIHM have a good effect in improving the energy metabolism and for treatment of mitochondria-related diseases, and the Qi-invigorating representative prescriptions Sijunzi Decoction (SD) was used for treatment of Qi deficiency, which is the object of study. The mechanism of energy metabolism improvement has been explored from mitochondrial oxidative phosphorylation, intracellular adenylates levels, and the mechanism of mitochondrial protection of SD were investigated. In summary, SD was able to improve mitochondrial function by enhancing cellular bioenergetics and had the pharmaceutical activities of mitochondrial protection. The study provides scientific evidence for the mechanism of Qi-invigoration in TCM which is achieved by improving mitochondrial energy metabolism.

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