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Review

Decline of life's energy theory of ageing 1. Revitalisation of energy metabolism and ageing mitochondria

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This article discusses the decline in mitochondrial energy production capability that occurs as animals age. Correlation is made between age-related mitochondrial functionality decline, lower growth hormone (GH) secretion and declining mitochondrial component levels and the chronic wasting conditions that arise as a consequence. During the past few years a number of patents have been issued that have utilised compositions containing the nutraceuticals carnitine, acetyl carnitine and their derivatives to therapeutically treat problems of energy metabolism. The administration of carnitine derivatives will improve the quality of living during the period of life when the body's energy declines. The significance of the patented therapeutic use of carnitine derivatives to re-establish mitochondrial functionality and its relationship to the ageing process are reviewed.

Keywords: *acetyl-L-carnitine, age, ageing, cardiolipin, carnitine, energy metabolism, growth hormone, mitochondria*

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1. Introduction

Mitochondria are the energy producing organelles that exist in every cell of the body. The energy created by mitochondria is in the chemical form of ATP. The sum composite of all the integrated energies of cells of an organism at any given time can be considered the 'life-force' of that organism. As the organism senesces, there is a progressive decline of this life-force, i.e. a progressive decline of life's energy (DOLE). In humans beyond 30 years of age, life-force declines in an exponential descending spiral. The decrease in the body's energy is observed as a steady decline in the ability to perform work [1]. The loss of the ability of the cells of the body to produce energy forms the basis of this 'whole body physical energy' decline [107]. As a consequence, all people and animals experience age-related alterations in body composition. For example, as the body's cellular energy level declines lean body mass shrinks, adipose mass expands [2,3], muscle strength declines [4], neural and defence mechanisms fail and cells slowly but consistently lose the capacity to function. The degree to which this has occurred, the extent of the decline, is the body's 'DOLE status'.

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2. Ageing and energy metabolism

The industrialised world has made dramatic strides towards curing acute disease causes of death, but the chronic diseases of old age persist. In 1900, life expectancy was 47 years and only 2% of persons lived beyond 60. A century later (2000) nearly 80% of all people can expect to survive beyond 60, although average life expectancy is still only near 80. Additionally, only a few percent of all people in the industrialised world die before the age of 30, after this age the rate of death due to natural causes increases exponentially. However, only rarely do individuals live beyond 90. During this period of exponential death, as the body weakens, it becomes vulnerable to chronic wasting diseases such as cancer, cardiovascular conditions and both endocrine and immune dysfunction. These disease conditions are a reflection of the DOLE status. In contrast to the years of youth the body progressively loses its ability to maintain its functionality after the first third of life. The body neither effectively repairs cellular tissue damage nor protects itself from microbes and/or undesirable cellular proliferation. As a consequence, the body increasingly loses the ability to oppose and avoid death. In 1825, Gompertz developed his now famous equation, a mathematical description of the incidence of death during the second phase of life [5,6]. It is a simple exponential expression of increasing mortality rate with age produced by the age-related energy decline observed in humans after 30 years of age.

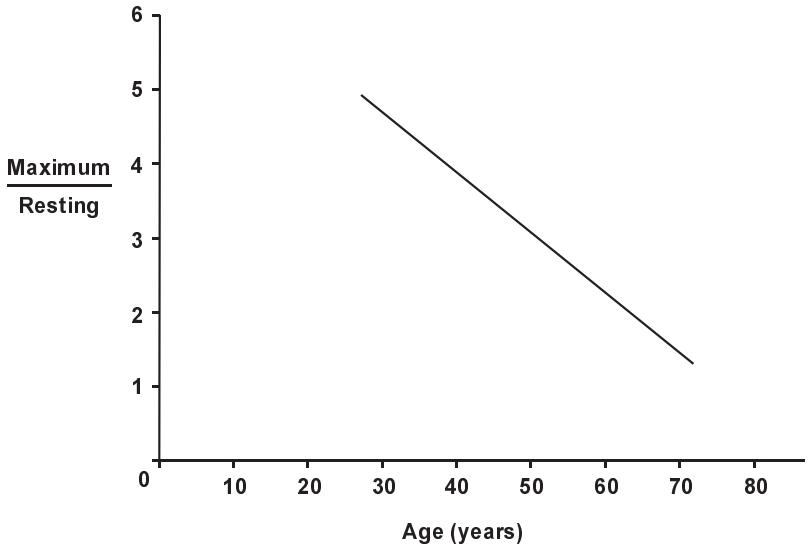
The metabolic mechanisms of ageing have not been satisfactorily explained by any single classical discipline. Comprehensive explanations of the decline of vitality extends beyond simply physiology and/or biology into physical chemistry, (electron transport mechanisms, free radical formation) [6-8], molecular biology, (fatty acid transport, membrane structure and chromosome replication) and mathematics (algorithms of endocrine interactions and cascades).

The 'rate of living' theory of ageing suggests that metabolic rate and longevity are inversely related and has been a viable explanation for more than half a century. The theory traces back to experiments of caloric restriction of rodents which were conducted starting in 1935 [9-11]. Restricting the caloric intake of the rodents by ~ 50% while maintaining an otherwise fully nutritionally complete diet, demonstrated that calorie restriction extended lifespan by 30 to 50%. However, the mechanism by which caloric restriction extends life has been elusive. Many studies have tried to correlate caloric restriction and a lower energy consumption with a lower basal metabolic rate (BMR). This would be analogous to running slower to facilitate running farther. Although there is an inverse correlation between BMR and longevity, critical exceptions exist. For example, rats and pigeons are about the same size and dimensions. However, the requirement for flight has significantly increased the BMR of the pigeons compared to rats, thus an expected decrease in the pigeon lifespan. The reality is that the life expectancy of pigeons is several times that of most rats [6,7]. Additionally, the nutritional modes (choice of diet) within genera of mammals affects both longevity and BMR [5,12]. Recently, an explanation for the longevity of caloric restricted animals has been offered in a new comprehensive theory of ageing, the hormonal imbalance-growth factor exposure theory (HI-GFE theory) [13-15]. Calorie restricted animals show markedly improved endocrine regulation that is maintained for an extended period as compared to animals fed *ad lib*. Improved hormone regulation in these animals promotes greater anabolism that stabilises cellular processes for a longer period.

The HI-GFE theory reasons that the loss of physiological functions in ageing animals is caused by an imbalance between the operational levels of two of the most prominent hormones in the body, GH and insulin. These two hormones are largely responsible for the maintenance of energy metabolism and cellular anabolic processes [13-15]. The loss of cellular functionality is due, not only to a decline in the rate of production of energy by mitochondria in the cells, but also to a reduced capacity of the mitochondria to produce energy (**Figure 1**).

Optimal mitochondrial energy production levels are necessary to maintain anabolic processes like protein and nucleic acid synthesis. Lower cellular energy that translates into lower anabolic rates also impact growth factor responses to regulatory signals. Old cells are clearly unable to generate energy at levels equal to those of younger animals across all species examined. The simple fact is, with age comes a loss of mitochondrial energy and this DOLE status makes the ageing body vulnerable to the chronic wasting diseases of cancer, arteriosclerosis, Alzheimer's, cardiovascular conditions and other age-related syndromes. [19-21,107]. The loss of mitochondrial energy producing capacity, particularly as it affects anabolic processes, may be considered one of the root causes

Figure 1: The declining capacity of mitochondrial respiration with age in human liver. The capacity to produce energy is measured by the mitochondria's respiration rates, in the presence (which decline with age) and absence (which are unaltered) of ATP precursors [20].



of the conditions of 'old age' [8,13-18] and a weak link in the maintenance of youthfulness.

The reduced ability of the cells to respond to both external and internal regulatory signals may be due to any one of several reasons. These reasons include: the inadequacy of the hormonal stimulation, the absence of receptor sensitivity, insufficient secondary messenger stimulation, intracellular component deficiencies and/or the insufficient response of available energy [13-15].

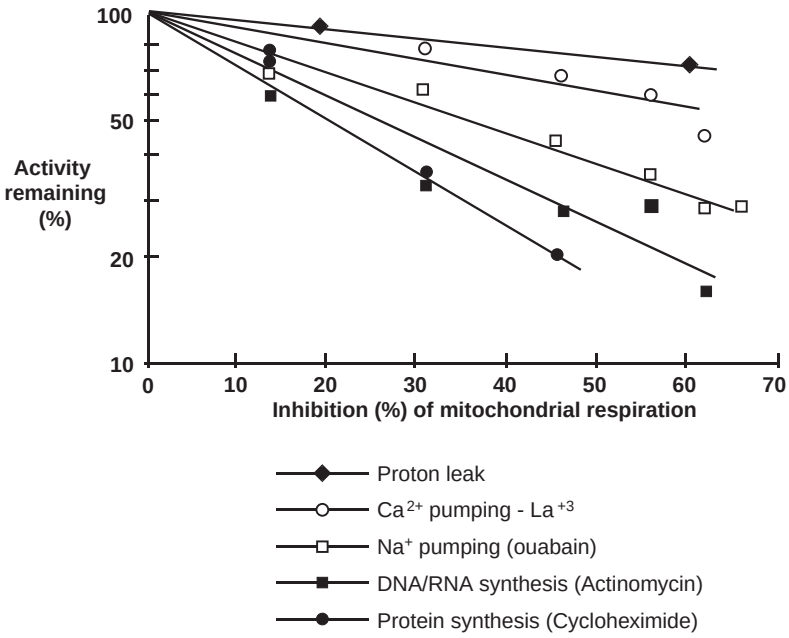
3. Age-related mitochondrial changes

The decline of mitochondrial energy production can be measured in changes in the mitochondrial membrane potential, membrane phospholipid and cardiolipin levels, the respiratory control ratio, electron transport enzymatic activities, the overall oxygen consumption and a reduction in electron transport efficiency. The loss of electron transport efficiency has the added detrimental effect of increasing the production of intracellular free radicals and enhancing oxidative damage [16,19,23,24].

Oxygen is used by the mitochondria in cells to produce the high energy compound ATP. ATP is used as an energy source for virtually every species of life on earth. The energy status of cells, tissues, organs and the whole body can be defined by the ability to produce and maintain threshold levels of ATP. The decline in ATP production is a significant impairment in old age [16], as can be seen by deductions from **Figures 1** and **2** that infer dramatic declines in protein synthetic capability with age. As a result anabolic activity in the body declines to catastrophic levels with age.

Age-related decrements in mitochondrial energy production result, at least in part, from changes in membrane cardiolipin levels, lipid composition and lipid-protein interactions [25-27]. These changes directly affect the activities of the enzyme systems that transport many critical small molecules within the mitochondria including adenine nucleotide, acyl-carnitine, pyruvate and phosphate [28-32]. The changes also directly influence the efficiency of the electron transport system and maximal energy producing capacity [33,34]. The mitochondrial inner membranes differ from membranes in other parts of the cell in that they contain phospholipids that have fatty acids with a higher degree of unsaturation, lower cholesterol and a higher cardiolipin content. Together with the insulin and GH status of the body, these factors impact the capacity of mitochondria to produce energy [35]. Cardiolipin in the mitochondrial membranes declines significantly with age [36-38], caused by either a lower biosynthetic rate, or because of oxidative damage which increases in ageing tissues [38,39]. In addition to lipids and cardiolipin, the levels of several other integral components of the mitochondria also decline with age. These include carnitine and its derivatives, which act as co-factors in the transport of fatty acids into the mitochondria for β-oxidation

Figure 2: The differential effects of the inhibition of mitochondrial respiration (Myxothiaxol) upon various cellular processes [32].



and ubiquinone (coenzyme Q^{10}), an electron transport component [40].

4. Growth hormone

The age-related alterations in body composition that become evident during adulthood include a progressive contraction in lean body mass and an increase in adipose tissue. These structural changes have been considered an unavoidable result of ageing [2,3,42]. The loss of lean body mass is the consequence of a lower ability to synthesise protein because of the lower level of energy production. There is a 60% decline in the maximum work output capacity between the ages of 30 and 70 years [1,4,41]. The atrophy of lean mass affects skeletal muscle, liver, kidneys, spleen, skin and bone and has been proposed to be the result of the reduced secretion and availability of GH [43-50,90,91].

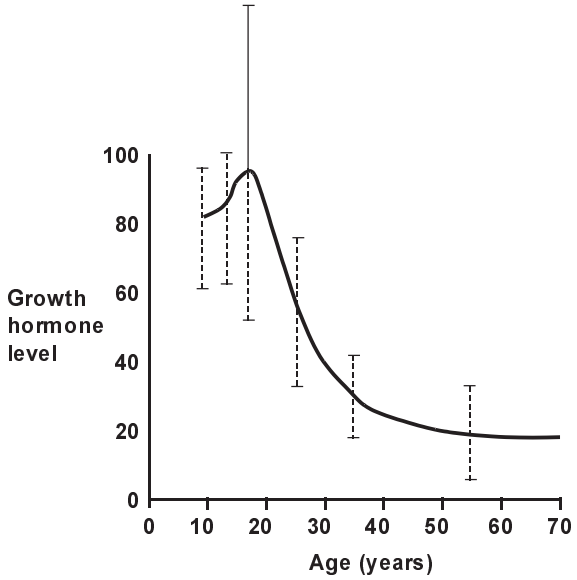
Although a number of hormones have been shown to influence mitochondrial function, GH is the most effective in restoring the age declined mitochondrial energy metabolism and membrane composition to normal [52-56]. The correlation of diminished GH secretion with increasing age is seen in all mammals studied (**Figure 3**) [58,59] including calorie restricted animals [60]. This does not mean a decreased capability of secreting GH with age. Under fasting conditions, older humans can secrete more than twice the GH as non-fasting young adults [59]; GH responsiveness to releasing hormone does not vary with age [61]. This is relevant to therapeutic treatment of the aged due to the significant tissue rejuvenating effects of exogenous GH in elderly humans [62,63].

One of the physiological effects of GH is to regulate the oxidative degradation of fatty acids (lipolysis) within mitochondria [23,64]. GH secretion occurs as a pulse during the early portion of human sleep; nocturnal rates of lipolysis are profoundly depressed by the absence of GH [65-71] and in ageing [68]. The loss of the pituitary gland lowers mitochondrial maximal energy production which is reflected in both decreased protein synthesis and mitochondrial protein turnover [70-72]. These changes are the consequence of increases in the degree of unsaturation and chain length of fatty acids producing softer, less rigid membranes and a 25 - 30% decrease in cardiolipin. In humans, functional and maximal mitochondrial energy production capacity is restored by daily GH injections. *In vivo* time course studies have shown that GH first changes the mitochondrial fatty acid composition, then improves respiration and energy production [35,52,55].

5. Cardiolipin

Cardiolipin is a phospholipid found almost exclusively in the inner membranes of mitochondria

Figure 3: The average levels of growth hormone as a function of age. The 24 h integrated (sum) as a percentage of an adolescent (= 100) [90].



and its integrity is critical for maximal energy production and the functioning of the enzymes of the electron transport system. Membrane functionality is determined by its composition. Although the composition of membranes varies with the quality and quantity of fat intake from the diet [73], the mitochondrial membranes function maximally only when the cardiolipin levels and the fatty acid composition is optimal [76].

A profound decrease in cardiolipin levels occurs during the course of the life of humans and animals [76,77]. This decrease parallels both a lower ratio of the maximal to basal mitochondrial energy producing capabilities (see **Figure 2**) [23,24] and a loss of inner mitochondrial membrane potential. For example, it was shown that a 75 year old woman had a 57% lower epidermal cardiolipin level relative to a 9 year old girl [74,75]. Calorie restricted animals, as compared to *ad lib*. fed animals, have an increased level of specific inner mitochondrial membrane unsaturated fatty acids as they age [81] that are instrumental to cardiolipin function [77]. The reduction of cardiolipin with increasing age is accompanied by a higher production of mitochondrial free radicals [64,79] and a lower level of anti-oxidant enzymes [78].

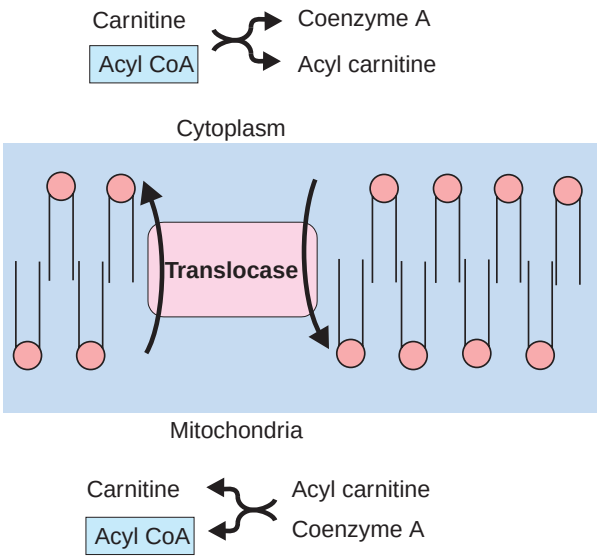
6. Acetyl-L-carnitine

Carnitine has two critical functions in the cell. Firstly, it facilitates fatty acid oxidation by acting as a co-factor in the transport of acyl groups across the inner mitochondrial membrane. Secondly, it functions to remove toxic acyl groups from the mitochondria and cell as esters. L-Carnitine and acetyl-L-carnitine (ALCAR) can be made endogenously or can be obtained from the diet. Together with acetyl CoA, they are essential co-factors in the steps of fatty acid transport through the outer membranes of the mitochondria. The steps shown in **Figure 4** are:

- Long chain free fatty acids are combined with CoA in the presence of ATP to form acyl CoA.
- The acyl-group of acyl CoA is esterified to carnitine forming an acyl-carnitine *via* the enzyme acyl-carnitine transferase-1.
- These acyl-carnitine esters are then transported into the mitochondrial matrix by carnitine translocase, a protein located within the inner mitochondrial membrane.
- Once in the mitochondria, the acyl-carnitine esters are converted back to acyl-CoA through the action of the enzyme, acyl-carnitine transferase-2.
- Acyl-CoA undergoes β-oxidation and the final product enters the Krebs cycle, which results in energy production [60].

A deficiency of L-carnitine interferes with the transport of fatty acids into the mitochondria producing an accumulation of both free fatty acids in the cytoplasm and acyl CoA within the mitochondria. The reduced level of fatty acids in the mitochondria

Figure 4: Diagram of the reactions of fatty acid transport across the mitochondrial membrane.



limits β-oxidation and yields lower energy production. As a result, the lower energy production translates into a lower level of protein synthesis in aged animals. Insufficient synthesis of muscle proteins lowers lean body mass. The fatty acids unused for energy production are stored as adipose tissue.

Pharmacological treatment of older animals with ALCAR improves overall mitochondrial function [101,105]. In aged animals the cardiolipin is one third lower than in younger counterparts, however, following treatment with ALCAR the mitochondrial status returns to normal [76,92]. These significant status reversals are due to the increases in cardiolipin content of mitochondrial membranes and activities of the mitochondrial enzymes [77]. Both cardiolipin levels and the higher frequency of unsaturated cardiolipin found in mitochondria of youthful animals [77,80] are restored by elevated ALCAR. These changes lead to higher transcription of the mitochondrial DNA, improved protein synthesis levels and improvements in functional capabilities of inner membrane bound mitochondrial enzyme systems, including fatty acid oxidation [76,77,80,81]. Carnitine stimulates the repair of mitochondrial membranes and returns functionality to mitochondria. Thus, the mitochondrial membranes that have changed over time are restored to the integrity to those of young animals by ALCAR [23,24,70].

7. Therapeutics

The therapeutic revitalisation of the body's energy supply has been the main thrust of a significant number of recent patents. Many of the patents teach the utilisation of compositions containing carnitine and its derivatives and/or Coenzyme Q^{10} to treat the symptoms of disease conditions. However, it is apparent that the pharmacological uses of these mitochondrial components have the 'whole body' effect of improving all cellular functions by stimulating energy productive capabilities. This cellular stimulation is facilitated by carnitine which upgrades both mitochondrial membrane maintenance and general energy metabolism and thereby all anabolic processes. L-Carnitine, and especially ALCAR, holds considerable potential as a therapeutic agent for the treatment of a variety of neural and muscle related conditions due to the high energy requirements of these tissues. Energy deficiencies are reflected in the chronic symptoms of the diseases of old age. These conditions include depression, chronic fatigue, attention deficit/hyperactivity disorder, ischaemic cardiac diseases [82], angina pectoris and hypotonia of skeletal muscles [107].

The plethora of patents teaching the uses of compositions containing carnitine and its derivatives address disease and metabolic conditions that arise as a consequence of changing metabolism. The symptoms treated with these compositions can generally be classed as deficiencies of the body's repair and

maintenance systems [105,107]. The most significant patents from a medical prospective (such as a remedy for cardiac ischaemia [105] or bone loss [121-123]) are those that are likely to be the least significant from an economic standpoint (such as hair restoration or weight loss [101-104,124,125]). Carnitine compositions are patented for the treatment of osteoarthritis [123] and osteoporosis [83-86,121,123], because of the ability of carnitine to stimulate mitochondrial reactivation and upgrade the anabolic regulation of the cells that manufacture the basal components of these tissues[116,117], bone (osteoblasts) [121,122] and cartilage (chondrocytes) [123]. Alleviating symptoms of various nervous conditions, anxiety, depression [108,109], chronic fatigue [110], attention deficit/hyperactivity disorder and even alcohol withdrawal symptoms [111,112] are claimed by other carnitine patents.

Possibly the most economically significant of the carnitine related patents concerns the use of compositions to control obesity [101] and pathological conditions of defective lipid metabolism. The improved mitochondrial energy metabolism not only burns fatty acids but also stimulates an increased protein synthesis that increases the body's lean mass. The change in lipid metabolism that results from carnitine administration leads to reduced hyperlipidaemia, alleviates high cholesterol conditions [102,103] and reportedly even reduces the appetite of obese patients [104].

Specific formulations can directly cause the catabolism of excess fatty acid utilising the revitalised mitochondria. The AMBI patented composition and its application [101] turns the mitochondria into an apparent furnace for burning fatty acids to promote fat and weight loss. The compositions of the patent are designed to cause/trick the mechanisms of the mitochondria to work without producing the normal quotient of energy as ATP. Together with carnitine derivatives the patented composition contains hydroxy-citrate and pyruvate. The unique composition uncouples the normal electron transport system's mechanisms of production of ATP from the oxidation of fatty acids. The ingenious process is simply designed to burn fat, without any other apparent benefits. However, it would appear that the process would produce a large amount of free radicals and consequently excessive cellular oxidative damage. Addition of a series of both lipotrophic and hydrophilic anti-oxidants would seem necessary to thwart the potential damage produced [113-115]

The consequences of improved energy metabolism teach several other patented uses for carnitine compositions. Specifically the epithelial benefits from carnitine administration are reflected in increased hair growth [124], hair follicles [125] and benefits to the condition of the patient's skin [119].

A recent patent submission [120] claims a technique of oral administration of a combination of the readily available nutraceutical ALCAR and the basic amino acid, L-ornithine, to elevate age declined GH release. The micromolar amounts of L-ornithine that were administered incrementally elevated the stimulated GH release to youthful levels and consequently the nexus of improved cardiolipin maintenance and elevated fat metabolism. This surprising effect seems to indicate a feed back relationship between the mitochondrial energy production capability and the levels of GH release. The modulation of age-related GH decline, either by injected GH, by GH secretagogues or using this new ALCAR/ornithine method reverses the decline in maximal mitochondrial energy production and the consequent declines in anabolic maintenance.

8. Expert opinion

The decline in energy production and energy producing capacity is a significant contributing factor to the chronic medical conditions that increase in parallel to the ageing process. In fact, in our opinion, it is one of the weakest links in the chain of life. Many medical conditions are alleviated by the stimulation of mitochondrial energy production by using, or by the administration of, simple carnitine-derived nutraceuticals. This realisation has generated a wide cross-section of recent therapeutic patents. The most likely economically impacting patents will be those used for weight control and to reverse obesity and its related problems such as cardiovascular, diabetic and other conditions. However, the most exciting impact is the use of the energy revitalisation compositions to enhance anabolic processes and reverse age-related functionality decline. The age-related conditions such as osteoporosis, loss of skeletal muscle strength (loss of lean body mass), increase in adipose mass, osteoarthritis, hair loss and loss of skin tone all respond to an improvement in the body's energy metabolism. As a consequence the utilisation of carnitine and its derivative will lead to an improvement in the quality of living during the later stages of life by revitalising the

mitochondria and reversing the declining level of the body's energy metabolism.

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Patents

Patents of special note have been highlighted as:
• of interest
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101. AMBI, INC.; PURCHASE, NY: US5914326 (1999).
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102. SIGMA-TAU IND. FARM RIUNITE SPA: WO9901126 (1999).

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•• Completely discusses the basis of carnitine supplementation and energy metabolism.

108. SIGMA-TAU HEALTHSCI SPA: WO9966914 (1999).

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111. SIGMA-TAU IND. FARM RIUNITE SPA: WO9917623 (1999).
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112. SIGMA-TAU IND. FARM. RIUNITE SPA: WO9952517 (1999).

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