The present invention relates to a method of increasing the intracellular concentration of phosphate by employing 2-(carbamimidoyl)-methyl-aminoethoxyphosphonic acid as vehicle and method to transport Phosphate into the cell. The increase in intracellular phosphate increases the availability of Adenosine Triphosphate and phosphocreatine, thereby leading to an increase in the anaerobic energy supply resulting in longer endurance and more forceful muscular contractions.
METHOD OF INCREASING INTRACELLULAR CONCENTRATIONS OF PHOSPHATE AND INCREASING THE FORCE OF MUSCULAR CONTRACTIONS

Field of the Invention

The present invention relates to a method of increasing the intracellular concentration of phosphate. Moreover, the increase in intracellular phosphate increases the availability of Adenosine Triphosphate (ATP) and phosphocreatine, thereby leading to longer endurance and more forceful muscular contractions.

Background

Phosphate, or phosphorus, is the second most abundant mineral in the body with calcium being the most abundant. As a Phosphate salt with calcium, Phosphate is involved in the formation of bone and teeth. In other salt complexes, Phosphate is involved in acid-base balance. Phosphate is also important for the structures of DNA and cell membranes however, one of the most important roles of Phosphate is energy production in muscle as ATP and Phosphocreatine. Phosphate is also part of a compound in red blood cells known as 2, 3 DPG (2, 3-diphosphoglycerate), which facilitates the release of oxygen to the muscle tissues.


It has been noted that 86% of body's supply of phosphate is stored in the bone, 14% exists in the in the somatic cells and only 0.3% existing in the extracellular space. Therefore, with such small amounts of phosphate existing in the somatic cells and extracellular space the supply of phosphate in the body can be rapidly depleted during periods of strenuous muscle contraction or muscular loads.
Natural phosphate enhancement can be achieved through diet and supplement consumption. However, because the majority of phosphate within a body is stored in the bone, an increase in phosphate, which is useful for cellular energetics through diet or direct supplementation alone, provides little if any increase in available phosphate to the somatic cells and extracellular space. Without an increase of the intracellular concentration of phosphate the energy required for muscle contraction will be quickly exhausted during physical activity. The energy requirements of contracting muscles involved in high-intensity exercise may increase 100-fold relative to resting muscles, exceeding the aerobic energy production capacity of the cells (Westerblad H, et al. News Physiol Sci. (2002) Feb;17:17-21). In this case anaerobic metabolism will provide additional energy. However, high-intensity exercise results in an eventual reduced capacity for muscle contractile function, or fatigue. Thus, there is seemingly a link between anaerobic metabolism and fatigue.

In a 2000 review on the role of creatine in skeletal muscle, Casey and Greenhaff provide a thorough overview of energy supply and utilization in muscle (Casey A, et al. Am J Clin Nutr (2000) Aug;72(2 Suppl):607S-17S). Adenosine Triphosphate (ATP) is the direct energy source for contracting muscle as energy for muscle contraction is released from the dephosphorylation of ATP to yield Adenosine Diphosphate (ADP) and inorganic phosphate (HPO$_4^{2-}$ or PO$_4^{3-}$ or Pi) in the following reaction:

$$\text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{Pi} + \text{H}^+ + \text{energy} \quad \text{(reaction 1)}$$

Therefore, the function of muscle is largely dependent on the availability of ATP. However, the concentration of ATP available in muscle at rest prior to the start of exercise is only enough to supply about 1-2 seconds of intense activity. ATP can be readily regenerated through the anaerobic dephosphorylation of available phosphocreatine. However, like ATP, the concentration of phosphocreatine in muscle is low and only enough to sustain muscle activity for about another 6 seconds. After repeated bouts of contraction, muscle phosphocreatine levels become nearly depleted (Greenhaff PL, et al. J Physiol. (1993) Jan;460:443-53). Fatigue, although likely multifaceted in terms of biochemical events, is the point at which the energy required by
contracting muscle exceeds the level available either from the stored supply of ATP or the indirect synthesis of high-energy ATP through phosphocreatine dephosphorylation.

The enzyme Creatine Kinase (CK) catalyzes the following reaction to regenerate phosphocreatine:

\[
\text{ATP + creatine} \leftrightarrow \text{ADP + phosphocreatine + H}^+ \quad \text{(reaction 2)}
\]

Reaction 2 is reversible depending on the energy state of the cell. In fast-twitch skeletal muscles, a large pool of phosphocreatine is available for immediate regeneration of ATP hydrolyzed during short periods of intense muscle contraction. Due to high CK activity in these muscles, the CK reaction remains in a near-equilibrium state, keeping the concentration of [ADP] and [ATP] almost constant over several seconds at the expense of phosphocreatine.

As can be noted from examination of reactions 1 and 2, a requirement of the regeneration of both ATP and phosphocreatine is a phosphate. Supplemental phosphate counters the chemically-induced reduction of ATP in rats (Rawson NE, et al. (1994) Jun;266(6 Pt 2);R1 792-6) and improves athletic performance in humans concomitant with increased cardiac function and aerobic capacity (Kreider RB, et al. Int J Sport Nutr. (1992) Mar;2(1):20-47). Thus, ensuring adequate supply of intracellular, intercellular, extracellular and intra-tissue phosphate may decrease the reliance on anaerobic metabolism to regenerate ATP, thereby allowing ample regeneration of phosphocreatine stores, even during times of strenuous physical activity.

Therefore there is a need to supply the body with intracellular, intercellular, extracellular and intra-tissue supplemental amounts of phosphate to increase the availability of ATP and phosphocreatine, thereby aiding in periods when strenuous muscular contractions are desired.

**Summary of the Invention**

The foregoing needs and other needs and objectives that will become apparent for the following description are achieved in the present invention, which comprises, a method of increasing the intracellular and intra-tissue levels of phosphate in a mammal. Furthermore, the method of increasing phosphate concentrations within a given tissue, comprises the administration of 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid, wherein said 2-(carbamimidoyl-
methyl-amino)ethoxyphosphonic acid acts as a phosphate donor to increase the relative concentrations of adenosine ATP to that of ADP as well as phosphocreatine to creatine.

**Detailed Description of the Invention**

In the following description, for the purposes of explanation, numerous specific details are set forth in order to provide a thorough understanding of the present invention. It will be apparent, however, to one skilled in the art that the present invention may be practiced without these specific details.

The present invention is directed towards a method of increasing the amount of phosphate within a cell wherein the relative concentration of ATP to ADP is increased via the administration of 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid.

2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid is a phosphoric ester derivative of creatine. 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid has been shown to be well tolerated and without side effects (Melloni GF, et al. Arzneimittelforschung. (1979) 29(9a):1447-9). Early studies of 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid explored its use as a treatment for heart lesions and to restore reduced cardiac contractile function, particularly after hypoxia. (Godfraind T, et al. (1984) 34(9):968-72). 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid has been successfully used to improve cardiac parameters in patients with inadequate coronary blood flow (Barlattani M, et al. (1979) 29(9a): 1483-5).

In terms of the metabolism of 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid, it is known that 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid administration in humans
increases urine levels of creatinine, the end metabolite and degradation product of creatine and phosphocreatine, which diffuses out of cells for excretion by the kidneys (Mellom GF, et al Arzneimittelforschung. (1979) 29(9a): 1447-9). Creatinine is also formed by a spontaneously occurring reaction -

\[ \text{phosphocreatine} \rightarrow \text{creatinine} + \text{H}_2\text{O} + \text{Pi} \]  (reaction 3)

The detection of increased creatinine resulting from 2-(carbamimidoyl-methyl-amo)ethoxyphosphonic acid administration indicates that 2-(carbamimidoyl-methyl-ammo)ethoxyphosphonic acid may serve as a source of physiological creatine, which provides well-established benefits to muscle metabolism and athletic performance mainly through the regeneration of phosphocreatine. However, 2-(carbamimidoyl-methyl-amo)ethoxyphosphonic acid as a source of creatine alone still requires a phosphate to form phosphocreatine, which in turn is the direct energy source for muscle contraction. Naturally, this phosphate would have to be drawn from a pool of available phosphate within the body, thereby reducing the availability of phosphate within the body for use in other reactions.

Advantageously, 2-(carbamimidoyl-methyl-amo)ethoxyphosphonic acid contains within it both creatine and phosphate to necessitate the regeneration of phosphocreatine. Oral administration in test animals reveals that 2-(carbamimidoyl-methyl-amo)ethoxyphosphonic acid is optimally absorbed by the intestine up to about 60% at 48 hours (Marzo A, et al Chin Ter (1972) Sep 15,62(5):419-30). 2-(carbamimidoyl-methyl-amo)ethoxyphosphonic acid was also advantageously found to be stable in both alkaline and acidic solutions, which is desirable for oral administration in animals. Moreover, via in vitro testing, it has been suggested that 2-(carbamimidoyl-methyl-amo)ethoxyphosphonic acid is dephosphorylated to creatine to some degree in the kidney, intestine and liver, and less so in the blood and muscle. Therefore, oral administration of 2-(carbamimidoyl-methyl-amo)ethoxyphosphonic acid is expected to result in fractions of both intact 2-(carbamimidoyl-methyl-amo)ethoxyphosphonic acid and dephosphorylated 2-(carbamimidoyl-methyl-amo)ethoxyphosphonic acid reaching the muscle,
further signifying that intact 2-(carbamimidoyl-methyl-ammo)ethoxyphosphonic acid exerts some unique beneficial effects in addition to supplying additional creatine.

Clinical trials have shown that 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid has effects related to skeletal muscle performance similar to creatine. 2-(carbamimidoyl-methyl-ammo)ethoxyphosphonic acid has been shown to improve muscle development and increase the capacity to perform physical activity. In one study, hand strength was improved by 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid administration and while remaining unaffected in a placebo group (Nicaise J, Curr Ther Res Clin Exp. (1975) 17(6):531-4). In another study conducted in elderly subjects, it was found that 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid improved muscular performance (Cavahen U, et al. Clin Ther. (1974) 69: 215-223).

Thus the beneficial effects afforded by 2-(carbamimidoyl-methyl-ammo)ethoxyphosphonic acid is understood to be a two-fold scheme whereby 2-(carbamimidoyl-methyl-ammo)ethoxyphosphomc acid 1) serves as a source of phosphate through a dephosphorylated fraction; and 2) acts as intact molecule with unique activity compared to phosphocreatine.

The beneficial effects related to 2-(carbamimidoyl-methyl-ammo)ethoxyphosphonic acid that have heretofore been undocumented in relation to creatine alone, is in part due to the unique structure and properties of 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid i.e. the presence of the phosphate moiety and its sparring effects on depletion of the phosphate pool for energy. This may beneficially affect both resting levels of energy stores by ‘priming’ the muscles’ energy stores prior to activity and actively contracting muscles during periods of strenuous physical activity by more efficient regeneration of energy stores.

It is herein understood that the administration of 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid leads to an increase in the intracellular, intercellular, extracellular and intra-tissue concentration of phosphate as it has been shown to be absorbed well in the intestine following oral administration. 14C radiolabel studies of 2-(carbamimidoyl-methyl-
amino)ethoxyphosphonic acid were performed to determine the absorption and distribution of 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid in Gumea-Pig following intravenous, intramuscular and oral administration. Significant amounts of ^14C radio—labeled 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid was found in the skeletal muscle, indicating that it was in fact able to cross the intestinal wall and be transported into the muscle cells.

Moreover, oral administration of 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid allows for a more constant level in the body for about 15 hours following administration, which begins to decrease after 33 hours (Marzo A, et al, Arch. Int. Pharmacodyn. (1971) 192, 378-392) As shown by the aforementioned experiments, since 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid is absorbed into the cell, it can therefore act as a vehicle to move phosphate into a cell. As such, the phosphate can then be donated to high-energy molecules within the cell. The increase in intracellular, intercellular, extracellular and intratissue phosphate allows for the more rapid phosphorylation of ADP and creatine to the high energy bearing molecules to ATP and phosphocreatine as discussed above, during anaerobic conditions. Therefore, the resulting relative increase in ATP and phosphocreatine leads to longer muscular endurance as well as more forceful muscle contractions.

As discussed above, it is recognized that 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid acts as a vehicle to transport phosphate into the cells where it can then dissociate to form the high-energy molecule ATP or to phosphorylate creatine to phosphocreatine, which can then donate the phosphate to ADP to form ATP to be used in muscular contractions. Under conditions wherein phosphate is not supplemented, it would, naturally have to be drawn from a pool of available phosphate, thereby reducing the availability of phosphate for other reactions, such as cellular signaling. Furthermore, the available phosphate existing in the somatic cells and extracellular space is already a diminutive portion of the body's totally supply of phosphate.

In one embodiment of the invention, a portion of 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid is fine-milled. U.S. Provisional Application No. 60/776,325
entitled "Compositions and Method for Increasing Bioavailability of Compositions for Performance Improvement", which is herein fully incorporated by reference discloses a method of improving the absorption, palatability, taste, texture and bioavailability of compounds by increasing the solubility. The increased bioavailability of a compound or ingredients is achieved via a reduction in particle size using a "fine-milling" technique. Any acceptable fine-milling technique will result in the fine-milled particles having an average particle size of between about 50 nm to about 2 nm. The reduction in size of the particle increases the surface area-to-volume ratio of each particle, thus increasing the rate of dissolution, thereby improving the rate of absorption.

As used herein, the terms "fine-milled" and/or "fine-milling" refer the process of micromization. Micromization is a mechanical process which involves the application of force to a particle, thereby resulting in a reduction in the size of said particle.

As used herein, the term "particle size" refers to the diameter of the particle. The term "average particle size" means that at least 50% of the particles in a sample will have the specified particle size. Preferably, at least 80% of the particles in a given sample will have the specified particle size, and more preferably, at least 90% of the particles in a given sample will have the specified particle size.

Although the preceding specification describes how 2-(carbamidoyl-methyl-amino)ethoxyphosphonic acid may be employed as an energetic molecule by way of transporting phosphate into the cell where it can act as phosphate donor to ADP and creatine, it should not be construed as the only method whereby 2-(carbamidoyl-methyl-amino)ethoxyphosphonic acid may be employed as an energetic molecule. From consideration of the specification, those of skill in the art may determine other methods wherein 2-(carbamidoyl-methyl-amino)ethoxyphosphonic acid may act as an energetic molecule.
Claims

What is claimed:

1. A method of increasing the intracellular concentration of phosphate comprising the step of administering to a mammal 2-(carbamimidoyl-methyl-ammo)ethoxyphosphonic acid

2. The method of claim 1 wherein said mammal is a human.

3. The method of claim 1 wherein the intracellular concentration of phosphate is increased in the somatic tissues of a mammal.

4. The method of claim 3 further comprising, increasing the relative concentration of Adenosine Triphosphate in said tissues.

5. The method of claim 4 wherein said tissues are selected from the group consisting of skeletal muscle tissue, cardiac muscle tissue, adipose tissue, epithelial tissues, and nervous tissue.

6. The method of claim 4 wherein said increase in Adenosine Triphosphate concentrations allows said mammal to produce more forceful muscular contractions.

7. A method of increases muscle endurance comprising, increasing the intracellular concentration of phosphate wherein the intracellular concentration of phosphate is increased by, the administration of 2-(carbamimidoyl-methyl-ammo)ethoxyphosphonic acid to a mammal.

8. The method of claim 7 further comprising reducing muscle fatigue wherein the administration of 2-(carbamimidoyl-methyl-amino)ethoxyphosphonic acid increases the Adenosine Triphosphate level in a mammal.

9. The method of claim 7 wherein said mammal is a human.

10. The method of claim 1 wherein at least a portion of said 2-(carbamimidoyl-methyl-ammo)ethoxyphosphonic acid is fine-milled.
INTERNATIONAL SEARCH REPORT  
International application No.  
PCT/CA2006/001428

A. CLASSIFICATION OF SUBJECT MATTER

IPC: A61K 31/661 (2006.01) , A61P 21/00 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A61K 31/661 (2006.01) , A61P 21/00 (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Pubmed, Delphion, Canadian Patent Database: creatinol, creatinol-o-phosphate, COP, ATP, adenosine, phosphate

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>US 6 602 512 (Sigma-Tau HealthScience) 05-08-2003 (see column 1, lines 60-67, column 2, lines 1-9)</td>
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[X ] Further documents are listed in the continuation of Box C.  
[ ] See patent family annex.

* Special categories of cited documents
  
"A" document defining the general state of the art which is not considered to be of particular relevance
  
"E" earlier application or patent but published on or after the international filing date
  
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  
"O" document referring to an oral disclosure, use, exhibition or other means
  
"P" document published prior to the international filing date but later than the priority date claimed
  
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  
"Y" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  
"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  
"A" document member of the same patent family

Date of the actual completion of the international search  
2 February 2007 (02-02-2007)

Date of mailing of the international search report  
22 May 2007 (22-05-2007)

Name and mailing address of the ISA/CA  
Authorized officer

Canadian Intellectual Property Office  
Place du Portage 1, CI 14 - 1st Floor, Box PCT  
50 Victoria Street  
Gatineau, Quebec K1A 0C9  
Facsimile No.: 001-819-953-2476  
Karol Gajewski 819-934-6734
## Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claim Nos 1-10
   - because they relate to subject matter not required to be searched by this Authority, namely Claims 1-10, directed to a method for treatment of the human or animal body by surgery or therapy which the International Search Authority is not required to search. Regardless, this Authority has carried out a search based on the alleged effects or purposes/uses of the product defined in claims 1-10.

2. [ ] Claim Nos
   - because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically

3. [ ] Claim Nos
   - because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6 4(a)

## Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims, it is covered by claim Nos.

**Remark on Protest**

- [ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- [ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- [ ] No protest accompanied the payment of additional search fees.
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