# La creatina: dall'utilizzo sportivo alle nuove prospettive di impiego in campo nutraceutico

Piero Sestili

Nutrizione e sport: tra miti e verità
Ancona, 17 Ottobre 2015

# Creatina

**La creatina è da molti anni il supplemento nutrizionale più usato dagli sportivi di tutto il mondo, sia da sola che in formulazioni più complesse**

# Creatina ed Energia

**Effetti sulla forza e massa muscolare**

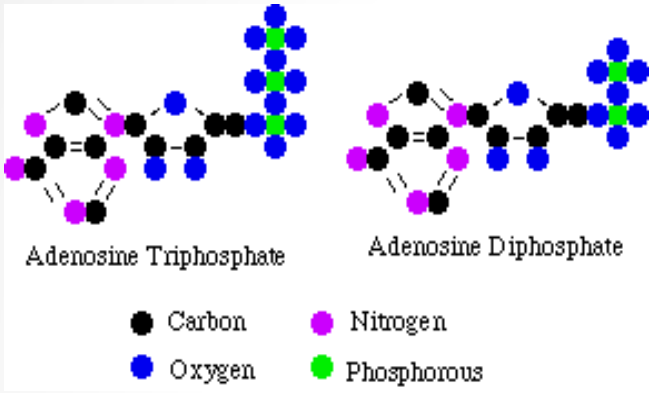
**Molti studi hanno esaminato e dimostrato gli effetti ergogenici della Cr, che produce un incremento di diversi parametri di forza, di resistenza e induce un certo grado di ipertrofia muscolare.**

**Dosi comprese tra i 5 ed i 20 g/die per almeno 21 giorni.**

# Creatina ed Energia

La creatina è uno dei depositi energetici fondamentali delle cellule ad alta richiesta energetica come quelle del muscolo, del cervello, dei reni e del pancreas.
Durante la contrazione muscolare la forma fosforilata della Cr, la fosfocreatina, rappresenta una fonte immedaita di gruppi fosfato ad alta energia, coi quali mantenere adeguati livelli di ATP.

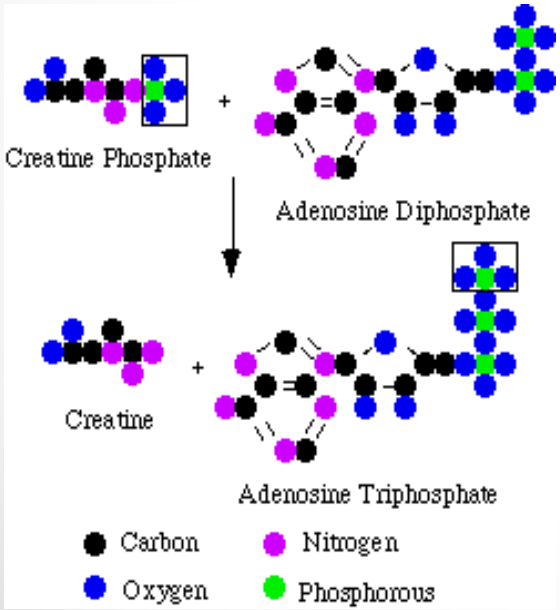
# Energetica della Contrazione: Le sorgenti di energia




*L'Adenosintrifosfato*

Una singola fibra muscolare "contiene" [ATP] ~ 4mM.

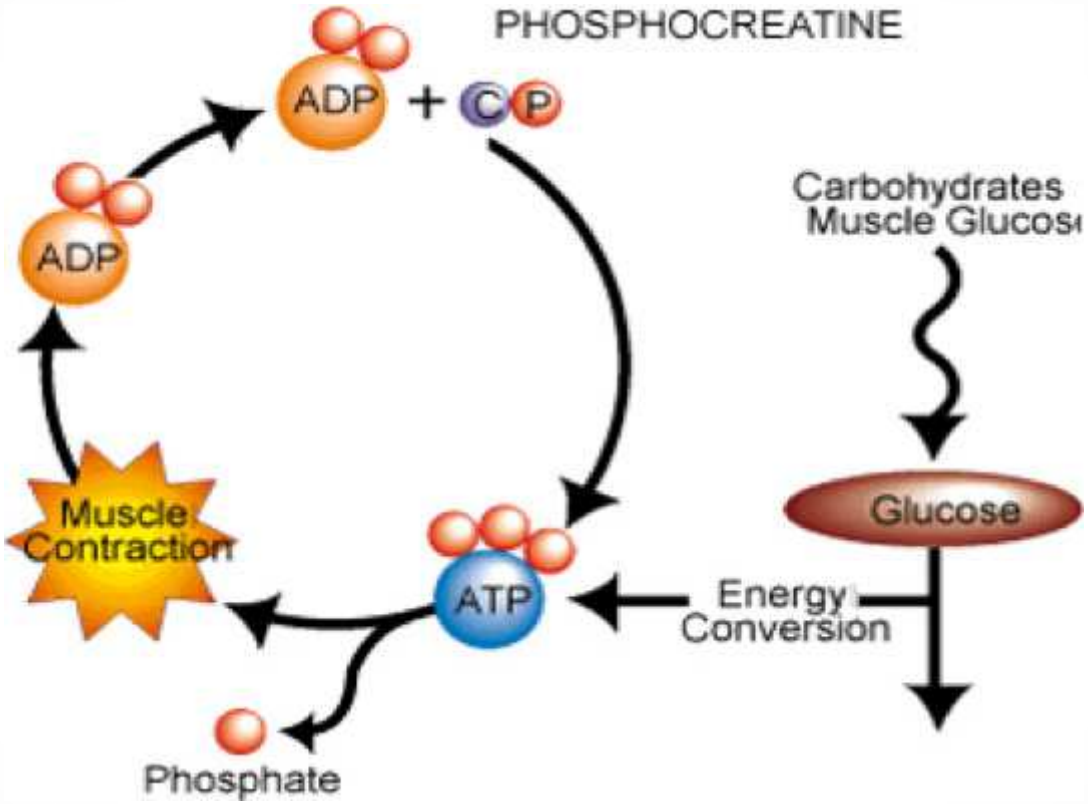
Questa quantitativo consente una contrazione completa per circa 1-2 sec.



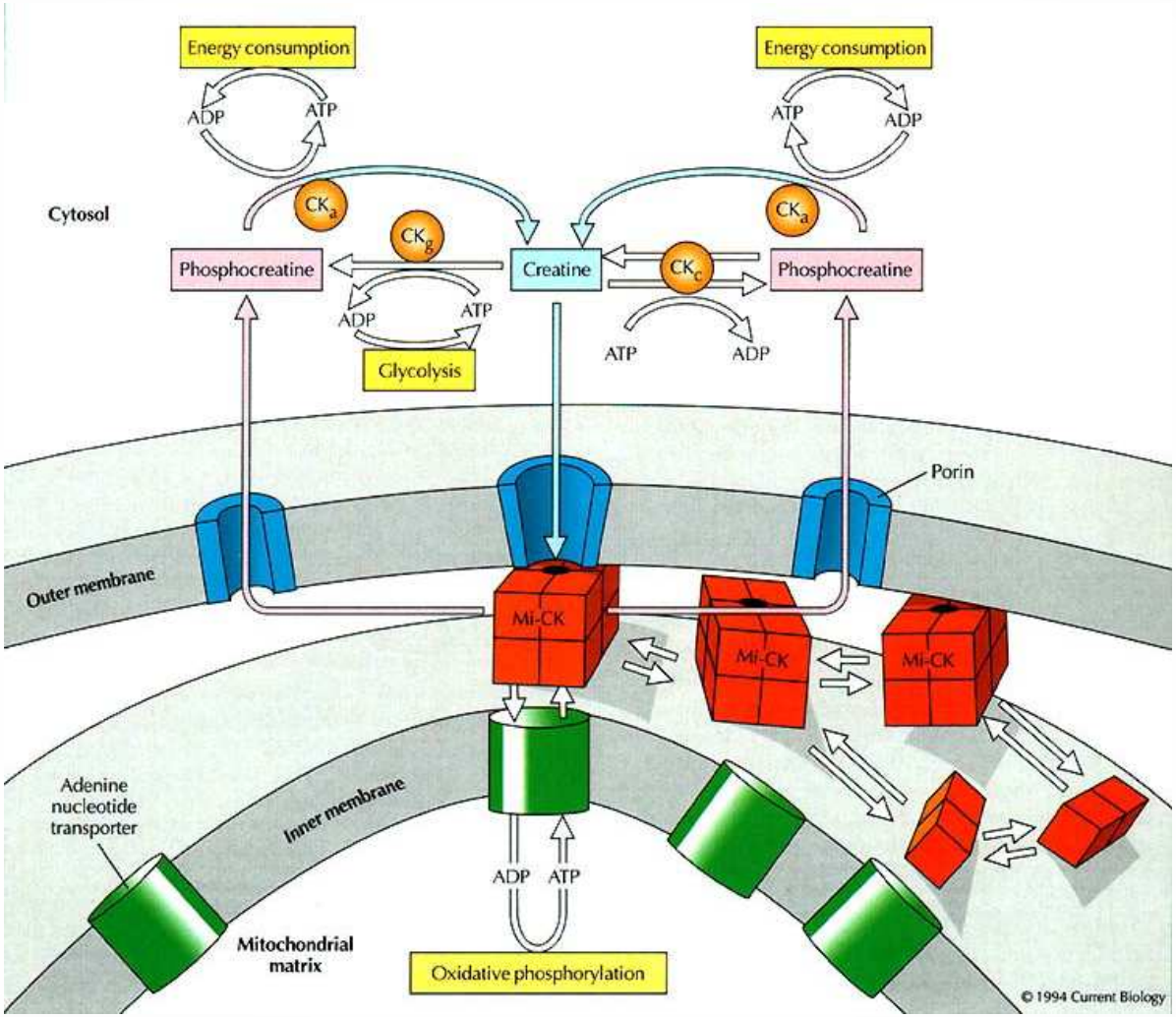

La *fosfocreatina* è la prima fonte di energia utilizzata dal muscolo, il suo quantitativo è circa 5 volte quello dell'ATP, cui cede un gruppo fosfato ad alta energia man mano che questo viene consumato (energy buffer)

La creatina costituisce una riserva rapida di ATP che può essere prontamente utilizzata dalla fibra

# Creatina ed Energia



# Creatina ed Energia

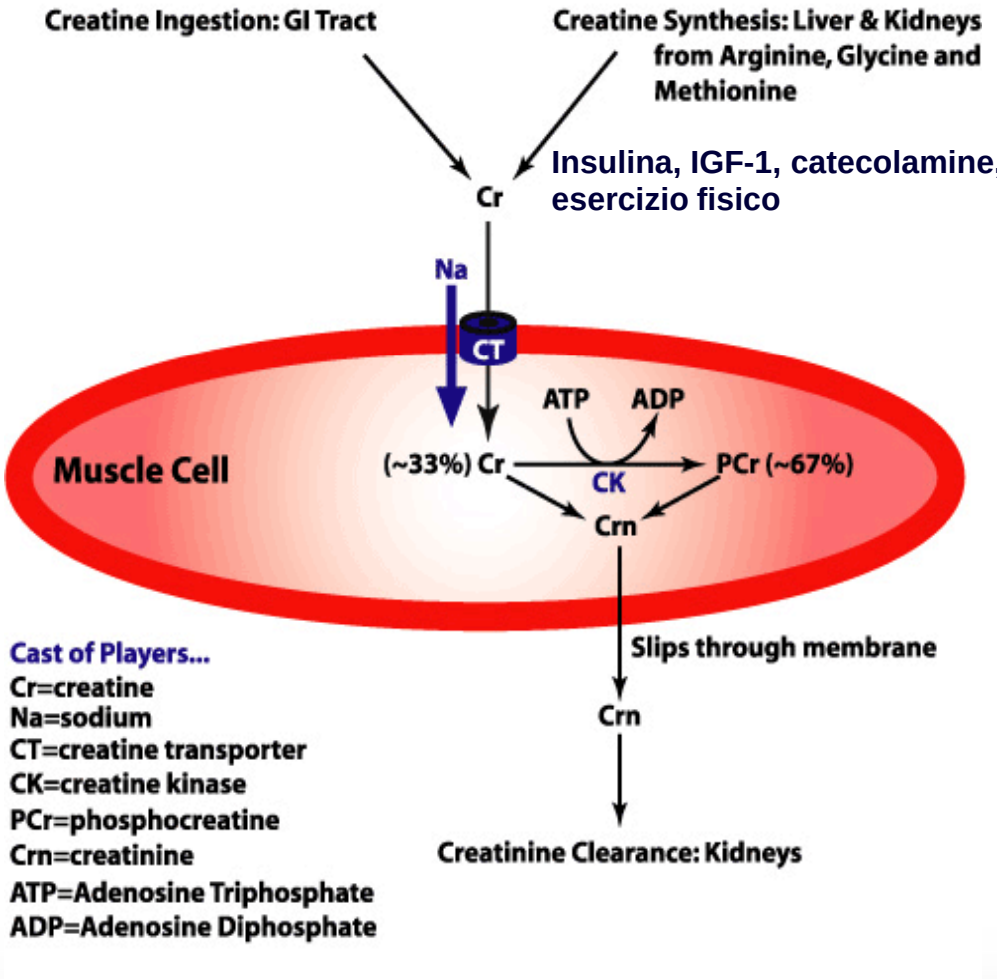


# Creatina ed Energia

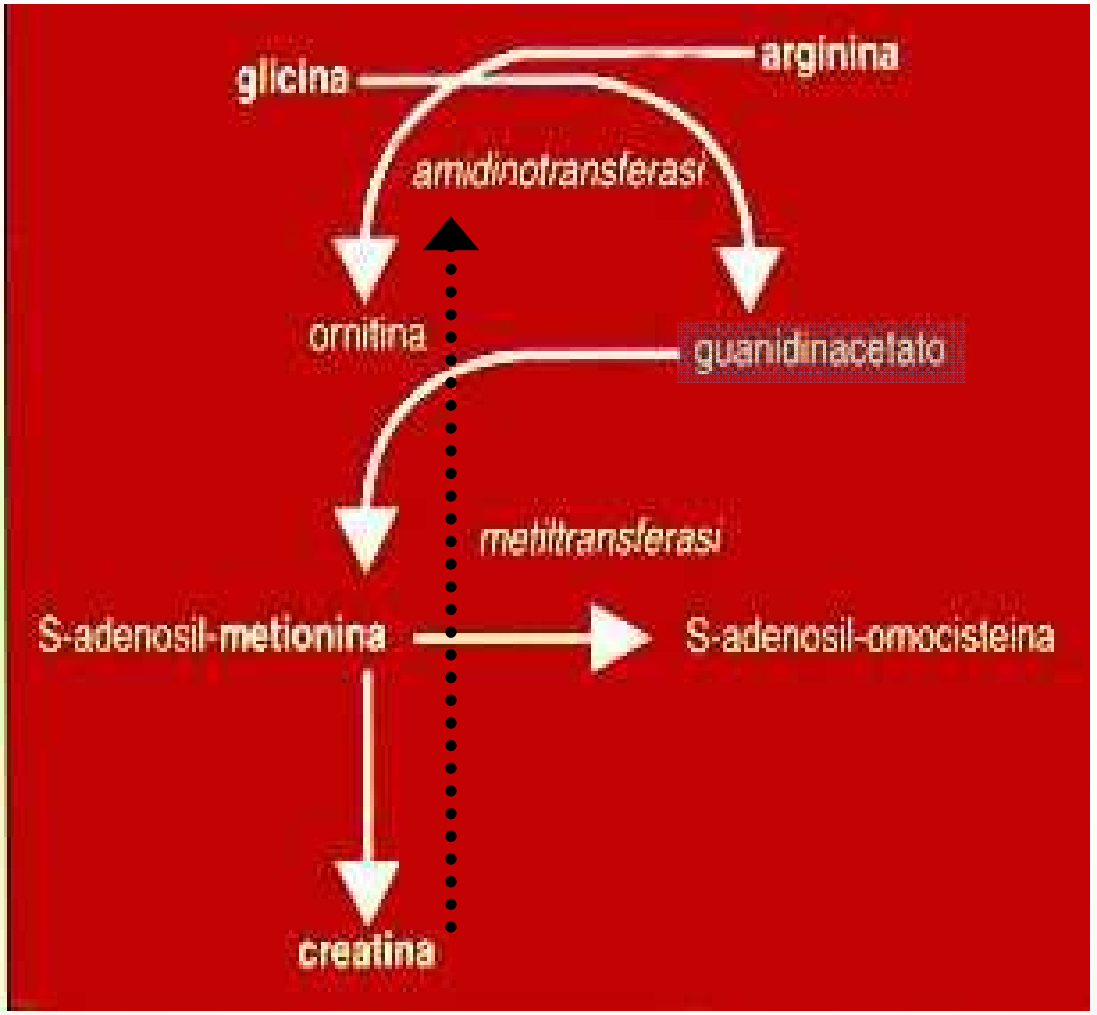
## Equilibrio della creatina

Il turnover giornaliero della Cr è pari a ca. 2g, ovvero i 2 g di Cr degradata irreversibilmente in creatinina, vengono rimpiazzati da 2g provenienti dalla dieta o dalla sintesi endogena (fegato, reni, pancreas). Esiste un bilancio ben regolato tra la Cr plasmatica e Cr intracellulare. Tale bilancio può essere modificato dall' ingestione di Cr ed in alcuni giorni la frazione intracellulare raggiunge un nuovo equilibrio.

# Trasporto della Cr nelle cellule



# Sintesi della Cr



# Fabbisogno e deficit di Creatina

La creatina, è evidentemente, molto importante per l'organismo, tanto che esso se ne assicura un livello basale "di sicurezza" attraverso delle **vie biosintetiche endogene** a partire dagli aminoacidi arginina e glicina: una extracerebrale, l'altra **cerebrale**.

# Fabbisogno e deficit di Creatina

Oltre a questo “**minimo sindacale**” la Cr viene assunta dalla dieta, con un “**ma**”:
solo **carne e pesce** ne contengono, i **vegetali no**.

# Creatina “extra”: quando?

**Supplementazione**

In clinica condizioni di deficit di Cr sono legate unicamente a patologie di origine genetica, che traggono beneficio dalla supplementazione con Cr orale.

Queste sindromi da deficit producono sintomi **per lo più a carico del SNC**, a sottolineare l’importanza non solo muscolare della creatina.

# Creatina “extra”: quando?

**Supplementazione**

Nello sport abbiamo già detto…e quindi avremmo detto tutto se non che…

# Creatina “extra”: quando?

**Supplementazione**

…l’individuazione di **nuove proprietà** della Cr che cooperano con il suo ruolo nell’energetica cellulare, ne sta però estendendo I possibili campi di utilizzo in medicina.

# Perché la creatina dovrebbe avere altre proprietà biologiche?

Ci sono altri fosfageni in natura?
Sì, ad esempio arginina fosfato svolge lo stesso ruolo della CrP nei molluschi. Ma nei vertebrati è sempre e solo la CrP a svolgere quel ruolo. A parte le caratteristiche cinetiche, perché l'evoluzione ha scelto la creatina?

… una spiegazione semplice potrebbe essere «perché a parità di prezzo offre di più!». Ma cosa?

| Table 1. Pro-differentiative and protective effect of creatine in vitro/in vivo | |
|---|---|
| **Skeletal Muscle** | |
| Cr enhances MRF-4 expression in human leg immobilization and rehabilitation | (Hespel et al. 2001) |
| Cr increases muscle strength and fibre size, MHC synthesis, myogenin and MRF-4 in in human skeletal muscle during heavy-resistance training | (Willoughby and Rosene 2001; Willoughby and Rosene 2003) |
| Cr reduces skeletal muscle necrosis and enhances mitochondrial function in Duchenne muscular dystrophic mice | (Passaquin et al. 2002) |
| Cr enhances fusion of satellite cells in vitro during differentiation | (Vierck et al. 2003) |
| Cr increases IGF-1 and myogenic regulatory factor (MRFs) in C2C12 cells and induces myotube hypertrophy | (Louis et al. 2004; Sestili et al. 2009) |
| Cr increases IGF-1 promoting muscle growth in human volunteers during resistance training | (Burke et al. 2008; Deldicque et al. 2005) |
| Cr enhances survival of C2C12 cells under hypertonic stress condition | (Alfieri et al. 2006) |

## Table 1. Pro-differentiative and protective effect of creatine in vitro/in vivo

| **Skeletal Muscle** | |
|---|---|
| Cr activates both p38 and Akt/PKB pathways promoting differentiation in C2C12 cells | (Deldicque et al. 2007) |
| Cr increases sphingosine kinase-1, PKB$\alpha$, p38 MAPK and ERK-6 promoting differentiation and muscle hypertrophy in human volunteers | (Safdar et al. 2008) |
| Cr induces the antioxidant enzyme peroxiredoxin-4, a type 2 peroxiredoxin-4 reductase and thioredoxin dependent peroxide reductase in C2C12 cells | (Young et al. 2010) |
| Cr protects the diaphragm from birth hypoxia (fibre atrophy, contractile dysfunction, and changes in mRNA levels of MuRF1 and myostatin) in mice | (Cannata et al. 2010) |
| Cr reduces oxidative DNA damage and lipid peroxidation induced by resistance exercise in male athletes | (Rahimi 2011) |
| Cr increases myotube diameters in C2C12 cells | (Ohira et al. 2011) |
| Cr inhibits lipoperoxidation and prevents GSH depletion in plasma and muscle of Wistar rat after exercise | (Deminice and Jordao 2012) |
| Cr reduces ROS content in muscles of Wistar rats | (Guimaraes-Ferreira et al. 2012) |
| Cr reverses myostatin-induced atrophy in myotubes modulating Akirin-1/Mighty mRNA expression in C2C12 cells | (Mobley et al. 2014) |

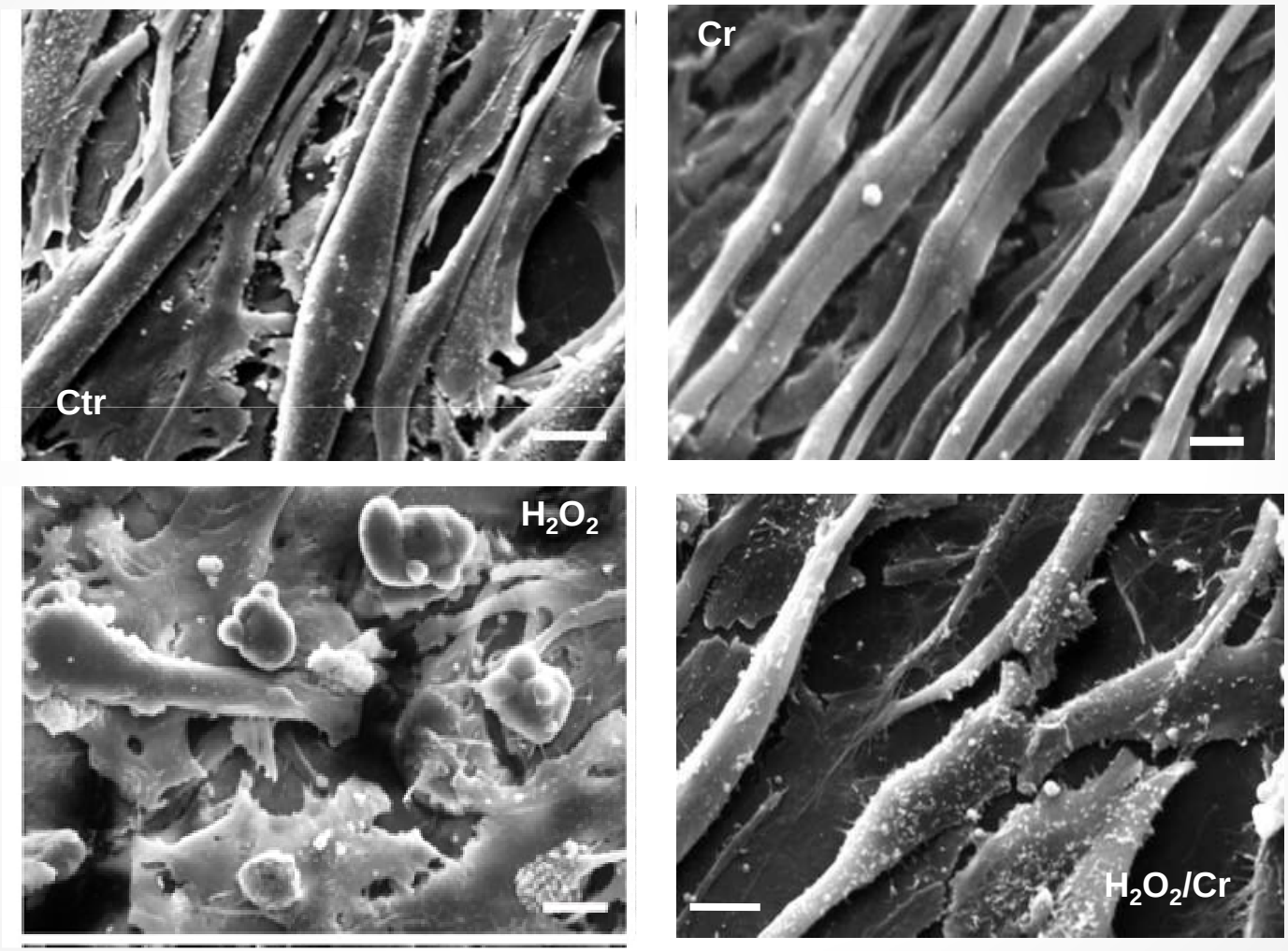
| Table 1. Pro-differentiative and protective effect of creatine in vitro/in vivo | |
|---|---|
| **Brain** | |
| Cr improves survival, slows the development of brain atrophy and reduces the formation of huntingtin-positive aggregates in mouse model of Huntington's Disease | (Ferrante et al. 2000) |
| Cr produces significant neuroprotective effects against NMDA mediated excitotoxic lesions in Sprague–Dawley rats | (Malcon et al. 2000) |
| Cr promotes differentiation of GABAergic neurons | (Andres et al. 2005; Ducray et al. 2007a; Ducray et al. 2007b) |
| Neuroprotection by long-term dietary creatine supplementation in G93 mice | (Pena-Altamira et al. 2005) |
| Cr and neurotrophin affect differentiation and/or survival of cultured fetal rat striatal nNOS-immunoreactive GABAergic interneurons | (Ducray et al. 2006) |
| Cr provides neuroprotection in hypoxic perinatal brain and increases the capacity of mice offspring to survive to birth hypoxia | (Ireland et al. 2011; Ireland et al. 2008) |
| Cr supplementation, combined or not with exercise, attenuates oxidative and neurochemical alterations against pentylenetetrazol-induced seizures in the rat cerebral cortex | (Rambo et al. 2013) |
| Attenuation of rotenone-induced mitochondrial oxidative damage and neurotoxicity in Drosophila melanogaster supplemented with Cr | (Hosamani et al. 2010) |

| Table 1. Pro-differentiative and protective effect of creatine in vitro/in vivo | |
|---|---|
| **Brain** | |
| Cr prevents lipoperoxidation, sulphydryls depletion and increases antioxidant capacity in phenylalan in Wistar rats' hippocampus caused by phenylalanin | (Berti et al. 2012) |
| Cr inhibits lipoperoxidation and protein oxidation in traumatic brain injury of Wistar rats | (Saraiva et al. 2012) |
| Cr promotes electrophysiological maturation and neurite length in differentiating primary chick neuroblasts and protects them from oxidative stress | (Sartini et al. 2012) |
| Cr induces differentiation of bone marrow stromal cells (from Sprague-Dawely rats) into GABAergic-like neurons | (Darabi et al. 2013; Mohammad-Gharibani et al. 2012) |
| Cr reduces brain infarct volume and increases serum IL-6 and decreases IL-18 concentrations in neonatal brains of female albino mice exposed to hypoxic ischemic insult | (Allahyar et al. 2015; Allahyar and Iqbal 2014) |
| Cr plus pyruvate supplementation to female rats during pregnancy and lactation prevents the deleterious effect of phenylalanine administration in offspring | (Bortoluzzi et al. 2014) |
| Cr promotes continuing propagation of rat inner ear-derived progenitor cells | (Di Santo et al. 2014) |
| Cr reduces ROS production and lipoperoxidation and prevents loss of mitochondrial membrane potential in the rat striatal silices exposed to 6-hydroxydopamine | (Cunha et al. 2013) |
| Maternal creatine supplementation improves the morpho-functional development of hippocampal neurons in rat offspring | (Ambrogini et al., in press) |

| Table 1. Pro-differentiative and protective effect of creatine in vitro/in vivo | |
|---|---|
| **Other tissues/cells** | |
| Cr protects from induction of the mtDNA common deletion generated in normal human fibroblast by ì UVA irradiation ì | (Berneburg et al. 2005) |
| Cr enhances alkaline phosphatase activity, differentiation and mineralization of osteoblast-like cells | (Gerber et al. 2005) |
| Cr protects mtDNA against UV- and oxidative stress-induced damage in human keratinocytes | (Lenz et al. 2005) |
| Cr affords cytoprotection in oxidatively injured cultured mammalian cells via direct antioxidant activity | (Sestili et al. 2006) |
| Cr prevents mitochondrial ROS formation through the preservation of mtCK activity in Wistar rat tissues | (Meyer et al. 2006; Santiago et al. 2008) |
| Cr improves health and survival in aged mice | (Bender et al. 2008) |
| Cr protects against oxidative mtDNA damage in HUVEC cells | (Guidi et al. 2008) |
| Cr protects against oxidative RNA damage in Jurkat cells | (Fimognari et al. 2009) |
| Co-supplementation of Cr and D-ribose prevents hypertrophy in hypoxic mice hearts and cardiomyocytes in vitro via AMPK and Akt | (Caretti et al. 2013) |

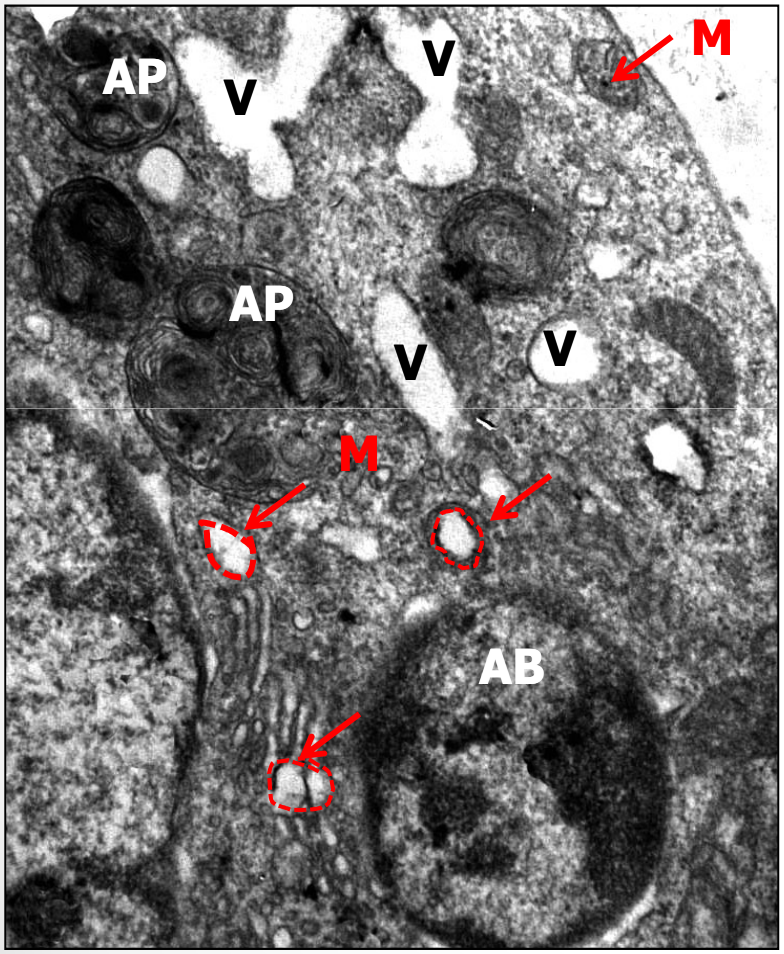
# SEM* of C2C12 at **Dif**ferentiating**D**ay5

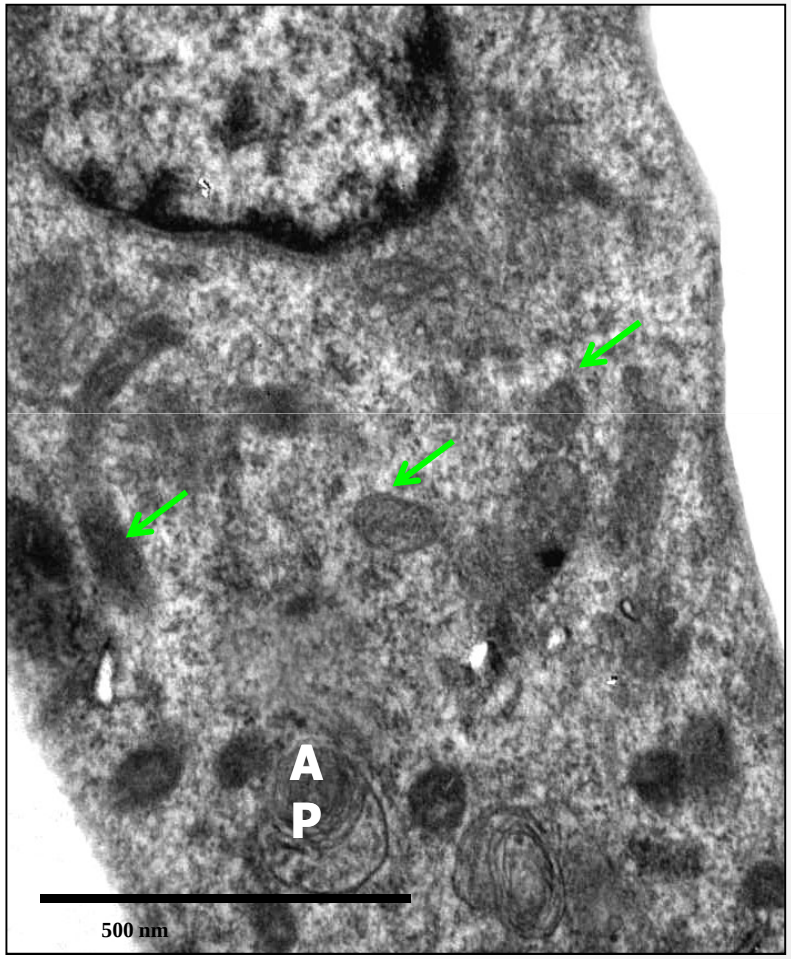
(miotubi in corso di differenziamento)




*microscopio elettronico a scansione

# TEM* at Dif D1: 0.3mM $H_2O_2$ - 1h w/wo Cr

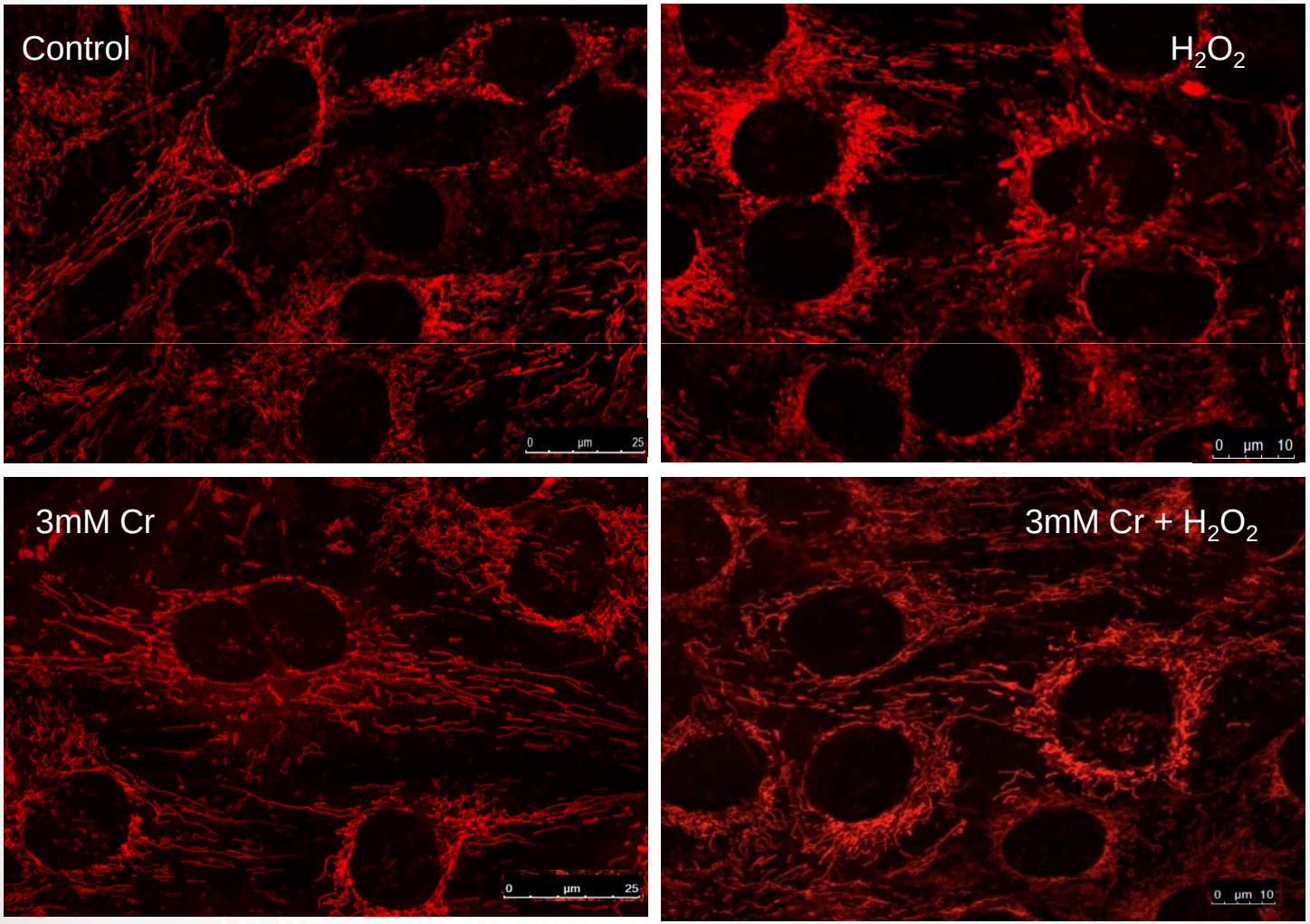



M: mitocondri, AP: autofagosomi, V: vacuoli

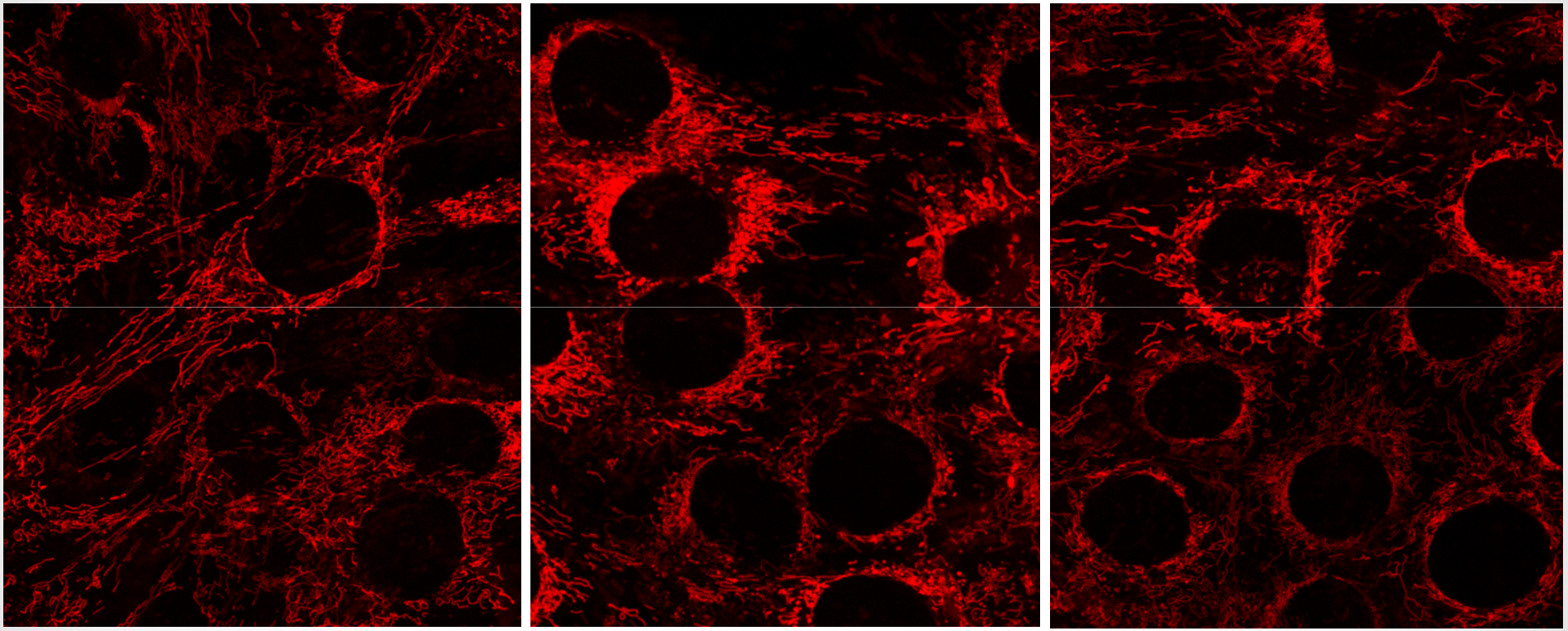
*microscopio elettronico a trasmissione


# Mitochondrial network




In post oxidative insult (3mM $H_2O_2$)

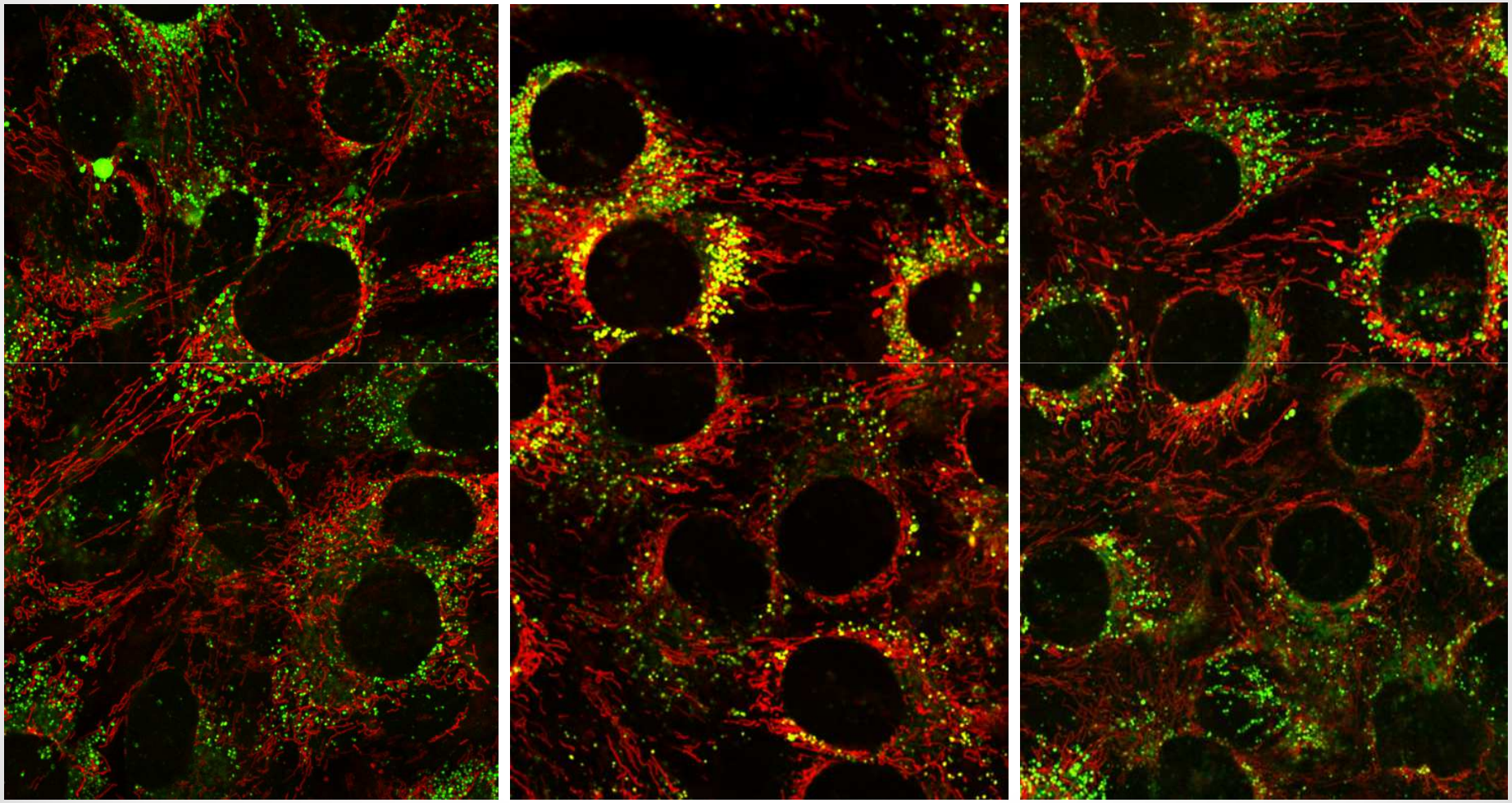
# Mitochondrial network



**Control** **$H_2O_2$** $H_2O_2$/Cr

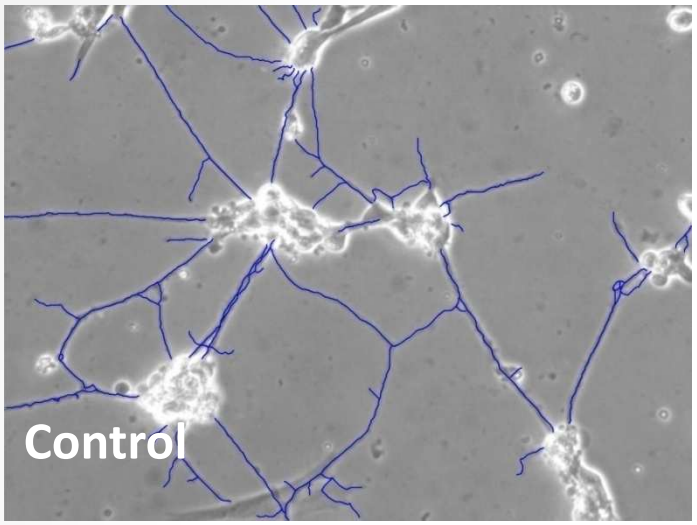
Dif D1

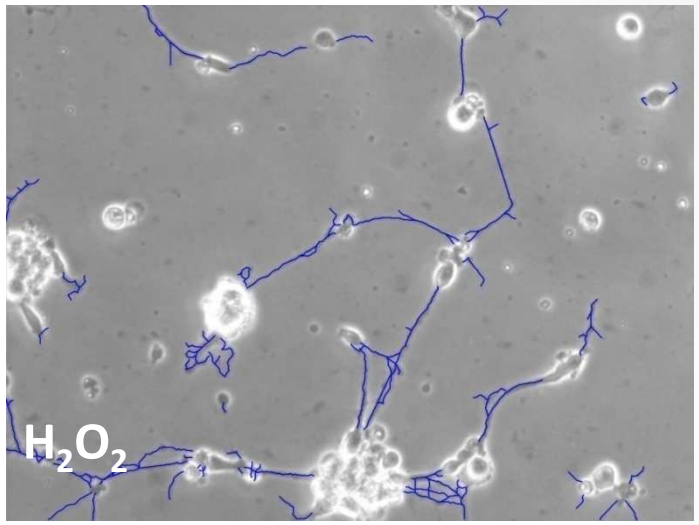

# Mitotracker red / Lysotracker green

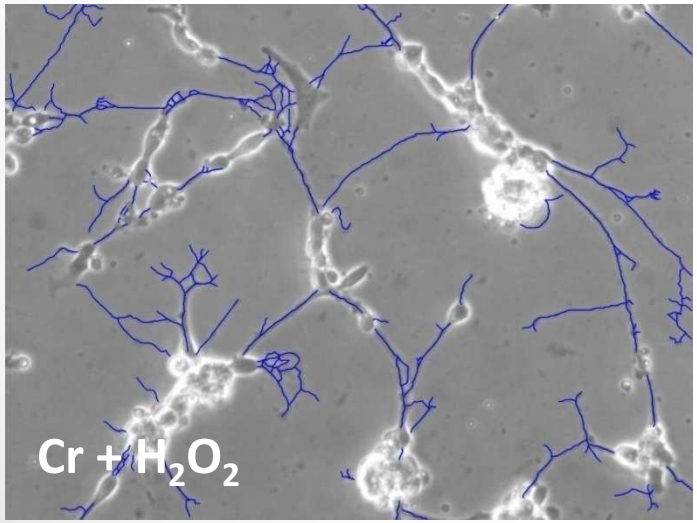


**Control** $H_2O_2$ $H_2O_2$/Cr

# Neurite length and branching in $H_2O_2$ –treated, DSCNs

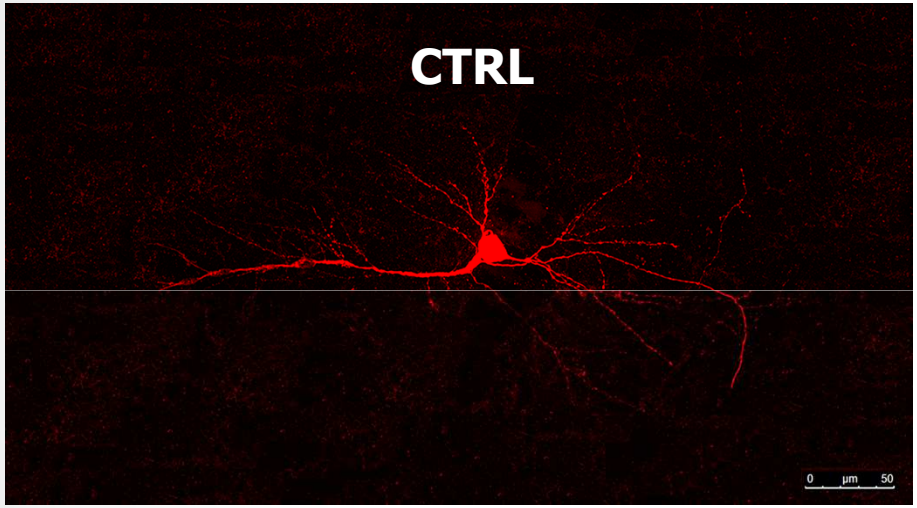


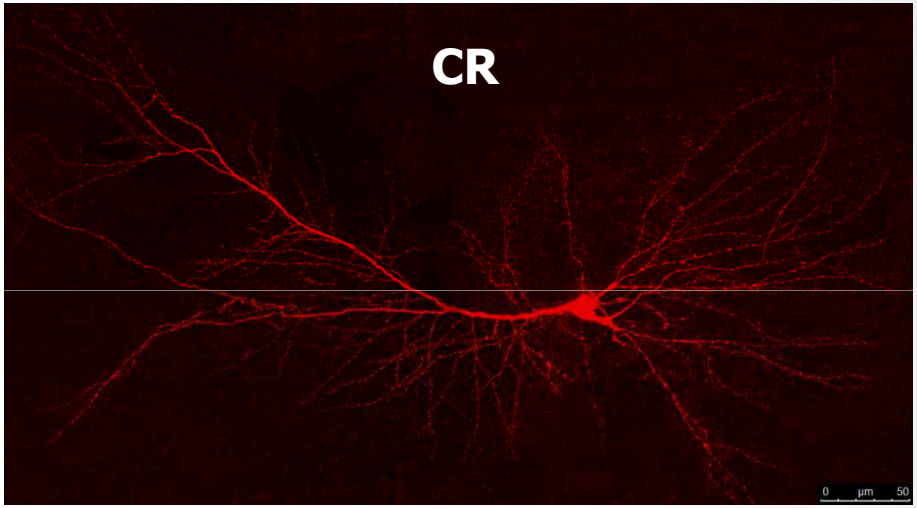



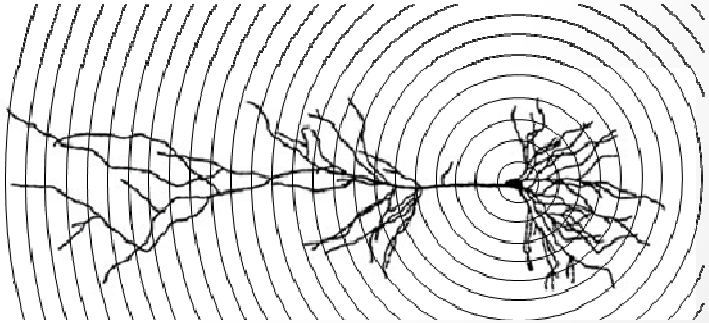

- **Image J – Neuron J macro analysis**

# Sholl analysis of biocytin loaded CA1neurons




Sholl analysis is a method of quantitative analysis commonly used in neuronal studies to characterize the dedritic  length and branching of an imaged neuron. Initial quantification of a neuron is performed by counting the number of dendrite intersections for concentric circles usually centered at the centroid of the cell body, of gradually increasing radius.



# Creatina come agente pleiotropico

- **Effetti prodifferenziativi** (mioblasti, neuroblasti, osteoblasti, cardiomiociti)
- **Effetti trofici** (muscolo scheletrico)
- **Effetto antiossidante diretto** (radical scavenging) **ed indiretto** (induzione di enzimi antiossidanti) **con spiccata localizzazione mitocondriale**
- **Protezione dei mitocondri e del DNA mitocondriale** (da insulto ossidativo e dei danni mitocondriali da UV)
- **Effetti citoprotettivi** (nei confronti di diversi tipi di stress chimici e fisici)
- **Effetto osmoprotettivo**

# Creatina come agente pleiotropico

Mol. Nutr. Food Res. 2009, *53*, 1187 – 1204 DOI 10.1002/mnfr.200800504

Research Article

**Creatine supplementation prevents the inhibition of myogenic differentiation in oxidatively injured C2C12 murine myoblasts**

Piero Sestili[1], Elena Barbieri[1], Chiara Martinelli[1], Michela Battistelli[2], Michele Guescini[1], Luciana Vallorani[1], Lucia Casadei[1], Alessandra D'Emilio[3], Elisabetta Falcieri[3,4], Giovanni Piccoli[1], Deborah Agostini[1], Giosuè Annibalini[1], Marco Paolillo[1], Anna Maria Gioacchini[1], and Vilberto Stocchi[1]

Journal of Neuroscience Research

**Creatine Affects In Vitro Electrophysiological Maturation of Neuroblasts and Protects Them From Oxidative Stress**

Stefano Sartini,[1] Piero Sestili,[2*] Evelin Colombo,[2] Chiara Martinelli,[2] Fanny Bartolini,[1] Stefano Ciuffoli,[1] Davide Lattanzi,[1] Davide Sisti,[1] and Riccardo Cuppini[1]

FINAL

*Send Orders for Reprints to reprints@benthamscience.ae*

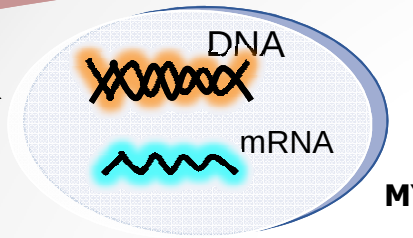
*Mini-Reviews in Medicinal Chemistry*, 2015, *15*, 000-000 1

**Effects of Creatine in Skeletal Muscle Cells and in Myoblasts Differentiating Under Normal or Oxidatively Stressing Conditions**

Piero Sestili*, Elena Barbieri and Vilberto Stocchi

# Creatine

MUSCLE CELL'S MEMBRANE




MYONUCLEI

**Oxidative stress**
**Osmotic stress**
**Cell death/apoptosis**

***Gene expression:***
**MRFs; IGF-1**

***Cell signalling:***
**AMPK; $Ca^{++}$;**
**AKT/mTOR; P38-MAPK**

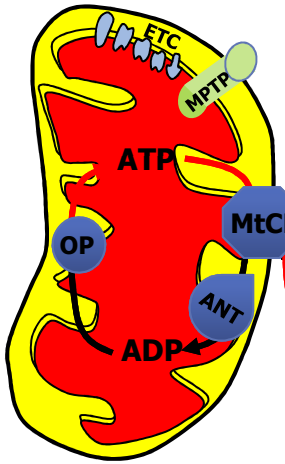
**Energy-related effects**
**Mitochondrial protection**
**mtDNA content/integrity**

**ROS scavenging, TrxR, GSH**

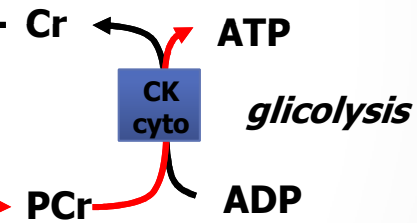
**Hypertrophy/protein syntesis**

**GLUT4 traslocation**
**Glucose uptake**

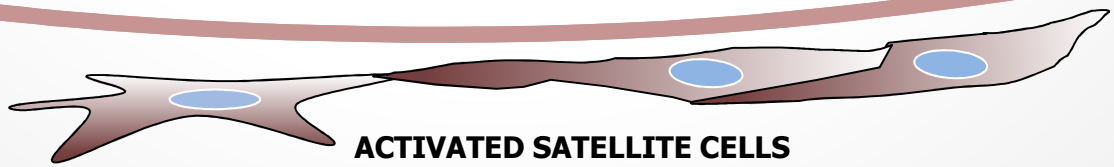
**Differentiation / Regeneration /Performance**




MITOCHONDRIA




MUSCLE CELL'S MEMBRANE



ACTIVATED SATELLITE CELLS

ELSEVIER

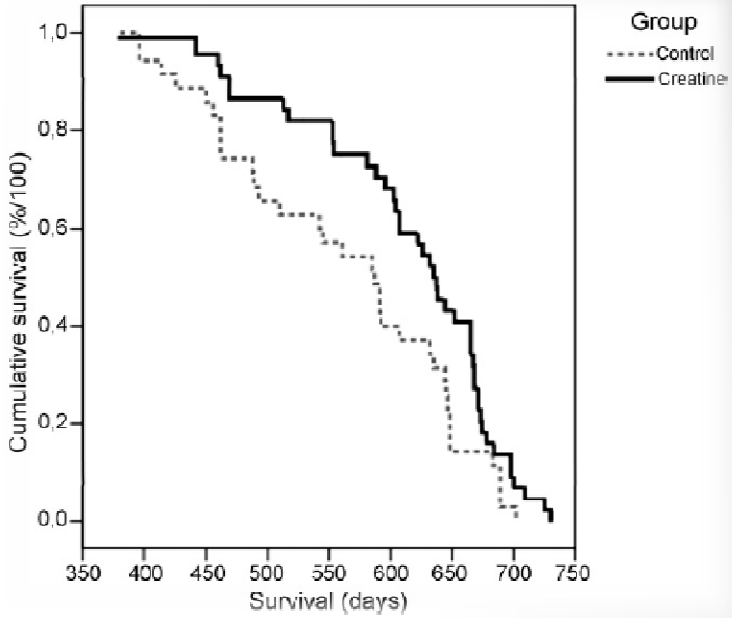
Neurobiology of Aging 29 (2008) 1404–1411

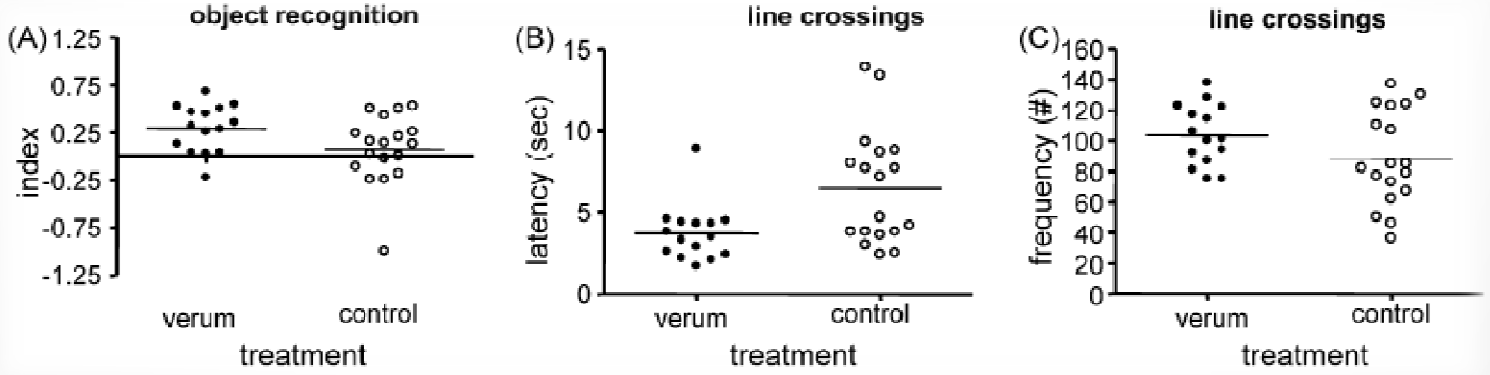
NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

# Creatine improves health and survival of mice

A. Bender[a], J. Beckers[b,c], I. Schneider[a,b], S.M. Hölter[b,d], T. Haack[a], T. Ruthsatz[e], D.M. Vogt-Weisenhorn[d], L. Becker[a,b], J. Genius[f], D. Rujescu[f], M. Irmler[b,c], T. Mijalski[b,c], M. Mader[g], L. Quintanilla-Martinez[h], H. Fuchs[b,c], V. Gailus-Durner[b,c], M. Hrabé de Angelis[b,c], W. Wurst[b,d], J. Schmidt[e], T. Klopstock[a,b,*]



ELSEVIER

Neurobiology of Aging 29 (2008) 1404–1411

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

# Creatine improves health and survival of mice

**Abstract**

The supplementation of creatine (Cr) has a marked neuroprotective effect in mouse models of neurodegenerative diseases. This has been assigned to the known bioenergetic, anti-apoptotic, anti-excitotoxic, and anti-oxidant properties of Cr. As aging and neurodegeneration share pathophysiological pathways, we investigated the effect of oral Cr supplementation on aging in 162 aged C57Bl/6J mice. Outcome variables included "healthy" life span, neurobehavioral phenotyping, as well as morphology, biochemistry, and expression profiling from brain. The median healthy life span of Cr-fed mice was 9% higher than in control mice, and they performed significantly better in neurobehavioral tests. In brains of Cr-treated mice, there was a trend towards a reduction of reactive oxygen species and significantly lower accumulation of the "aging pigment" lipofuscin. Expression profiling showed an upregulation of genes implicated in neuronal growth, neuroprotection, and learning. These data show that Cr improves health and longevity in mice. Cr may be a promising food supplement to promote healthy human aging.


*Keywords:* Creatine; Aging; Oxidative stress; Mitochondria; Lipofuscin; Life span

Amino Acids (2011) 40:1349–1362
DOI 10.1007/s00726-011-0855-9

REVIEW ARTICLE

# Use of creatine in the elderly and evidence for effects on cognitive function in young and old

**Eric S. Rawson · Andrew C. Venezia**

supplementation. Creatine is an inexpensive and safe dietary supplement that has both peripheral and central effects. The benefits afforded to older adults through creatine ingestion are substantial, can improve quality of life, and ultimately may reduce the disease burden associated with sarcopenia and cognitive dysfunction.

**Keywords** Dietary supplement · Ergogenic aid · Fatigue · Phosphocreatine · Aging

Amino Acids (2007)
DOI 10.1007/s00726-007-0508-1
Printed in The Netherlands

Amino Acids

# Effects of creatine supplementation on glucose tolerance and insulin sensitivity in sedentary healthy males undergoing aerobic training

B. Gualano, R. B. Novaes, G. G. Artioli, T. O. Freire, D. F. Coelho, F. B. Scagliusi, P. S. Rogeri, H. Roschel, C. Ugrinowitsch, and A. H. Lancha Junior

between groups or over time in fasting insulin or HOMA. The results suggest that creatine supplementation, combined with aerobic training, can improve glucose tolerance but does not affect insulin sensitivity, and may warrant further investigation with diabetic subjects.

CLINICAL SCIENCES

# Creatine in Type 2 Diabetes: A Randomized, Double-Blind, Placebo-Controlled Trial

BRUNO GUALANO[1,2], VITOR DE SALLES PAINNELI[1,2], HAMILTON ROSCHEL[1,2], GUILHERME GIANNINI ARTIOLI[1,2], MANOEL NEVES JR[2], ANA LÚCIA DE SÁ PINTO[2], MARIA ELIZABETH ROSSI DA SILVA[3], MARIA ROSÁRIA CUNHA[3], MARIA CONCEPCIÓN GARCÍA OTADUY[4], CLAUDIA DA COSTA LEITE[4], JÚLIO CÉSAR FERREIRA[1], ROSA MARIA PEREIRA[2], PATRÍCIA CHAKUR BRUM[1], ELOISA BONFÁ[2], and ANTONIO HERBERT LANCHA JR[1]

*[1]Laboratory of Applied Nutrition and Metabolism, School of Physical Education and Sports, University of São Paulo, São Paulo, BRAZIL; [2]Division of Rheumatology, School of Medicine, University of São Paulo, São Paulo, BRAZIL; [3]Division of Endocrinology Laboratory of Medical Investigation, School of Medicine, University of São Paulo, São Paulo, BRAZIL; and [4]Division of Radiology, School of Medicine, University of São Paulo, São Paulo, BRAZIL*

rable between the groups. **Conclusions**: Creatine supplementation combined with an exercise program improves glycemic control in type 2 diabetic patients. The underlying mechanism seems to be related to an increase in GLUT-4 recruitment to the sarcolemma.

**Key Words:** CREATINE SUPPLEMENTATION, EXERCISE TRAINING, TYPE 2 DIABETES, THERAPEUTIC EFFECTS

Dickinson *et al. BMC Pregnancy and Childbirth* 2014, **14**:150
http://www.biomedcentral.com/1471-2393/14/150

BMC
Pregnancy & Childbirth

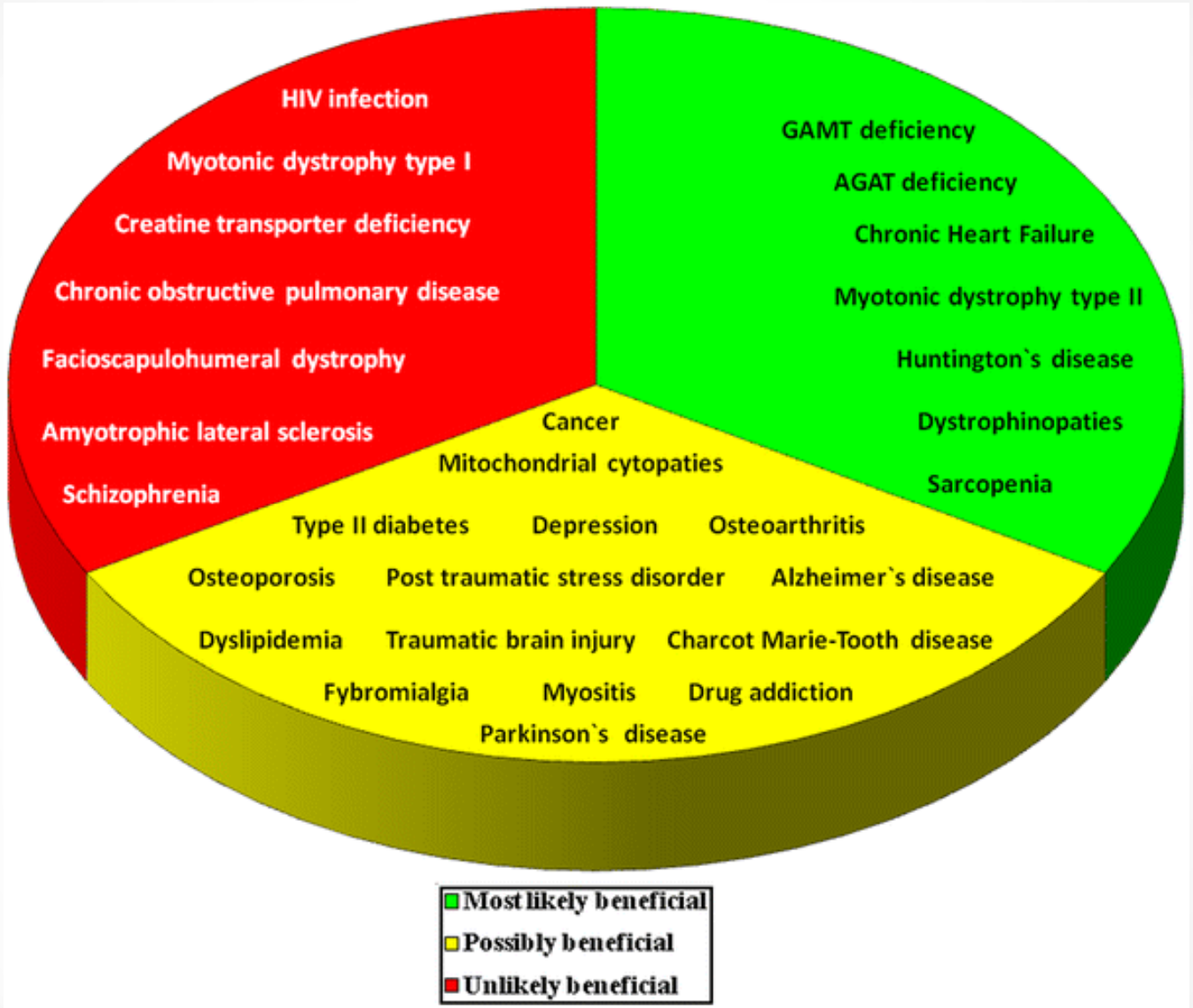
REVIEW Open Access

# Creatine supplementation during pregnancy: summary of experimental studies suggesting a treatment to improve fetal and neonatal morbidity and reduce mortality in high-risk human pregnancy

Hayley Dickinson[1], Stacey Ellery[1], Zoe Ireland[2], Domenic LaRosa[1], Rodney Snow[3] and David W Walker[1,4*]

**Abstract**

While the use of creatine in human pregnancy is yet to be fully evaluated, its long-term use in healthy adults appears to be safe, and its well documented neuroprotective properties have recently been extended by demonstrations that creatine improves cognitive function in normal and elderly people, and motor skills in sleep-deprived subjects. Creatine has many actions likely to benefit the fetus and newborn, because pregnancy is a state of heightened metabolic activity, and the placenta is a key source of free radicals of oxygen and nitrogen. The multiple benefits of supplementary creatine arise from the fact that the creatine-phosphocreatine [PCr] system has physiologically important roles that include maintenance of intracellular ATP and acid–base balance, post-ischaemic recovery of protein synthesis, cerebral vasodilation, antioxidant actions, and stabilisation of lipid membranes. In the brain, creatine not only reduces lipid peroxidation and improves cerebral perfusion, its interaction with the benzodiazepine site of the $GABA_A$ receptor is likely to counteract the effects of glutamate excitotoxicity – actions that may protect the preterm and term fetal brain from the effects of birth hypoxia. In this review we discuss the development of creatine synthesis during fetal life, the transfer of creatine from mother to fetus, and propose that creatine supplementation during pregnancy may have benefits for the fetus and neonate whenever oxidative stress or feto-placental hypoxia arise, as in cases of fetal growth restriction, premature birth, or when parturition is delayed or complicated by oxygen deprivation of the newborn.


HIV infection
Myotonic dystrophy type I
Creatine transporter deficiency
Chronic obstructive pulmonary disease
Facioscapulohumeral dystrophy
Amyotrophic lateral sclerosis
Schizophrenia
GAMT deficiency
AGAT deficiency
Chronic Heart Failure
Myotonic dystrophy type II
Huntington`s disease
Dystrophinopaties
Sarcopenia
Cancer
Mitochondrial cytopaties
Type II diabetes
Depression
Osteoarthritis
Osteoporosis
Post traumatic stress disorder
Alzheimer`s disease
Dyslipidemia
Traumatic brain injury
Charcot Marie-Tooth disease
Fybromialgia
Myositis
Drug addiction
Parkinson`s disease
Most likely beneficial
Possibly beneficial
Unlikely beneficial

# Conclusioni: creatina come, quando, quanto?

- **Nello sport**
- **Nella vita quotidiana, specie a partire dall'età adulta e nei vegetariani, ed in presenza di fattori di rischio quali intolleranza al glucosio**
- **Nell'anziano**
- **Nelle donne in gravidanza, a partire dal III trimestre**

# Una questione di purezza, nello sport e nella vita quotidiana

**Tre mesi di supplementazione con Cr per un atleta implica il consumo di un totale di 600 g, da cui, in base alla qualità del prodotto**

• • •

| | | |
|---|---|---|
| **Cr 99.8%** | **~** | **1,2 g impurezze** |
| **Cr 98.0%** | **~** | **12 g impurezze** |
| **Cr 95.0%** | **~** | **30 g impurezze** |