Supplements & Mitochondrial disease

Mito Information Day, Adelaide

9th Sep 2017
Christina Liang
Royal North Shore Hospital
Mitochondrial Cocktails
Supplements

• **How do we think about supplements**
  – Why do we take them?

• How we & others use supplements
  – Compared: Australia, US, Netherlands
    • What are we trying to treat?

• Current therapies and supplements
  – Supplements vs what are still in the pipeline

• Examples of some supplements
  – And how they might work
Why are we crazy about the supplements?

• Patients’ point of view
  • No specific or curative treatment

– Pros
  • To improve things
    – Over the counter
    – No prescription
  • Hope: the NEXT BEST THING NOW

– Cons
  • $$$
  • Tablets/ Powder/ Capsule burden
  • Information overload
Why crazy about the supplements?

- **Doctor’s point of view**
  - No specific or curative treatment

- **Pros**
  - Try something than do nothing
    - Low risk
  - Hope: “MAYBE something MIGHT WORK a little NOW”

- **Cons**
  - Unclear evidence – what to advise
  - $$$
  - Tablets/ Powder/ Capsule burden
Not enough data

- None shown to be clearly effective
  - Case reports
  - Open-label trials
  - Retrospective studies
  - Small-scale clinical trials

- As of 2013:
  - Of 1039 publications on treatment for mitochondrial disease
    - Only 35 included observations on > 5 patients
Supplements

• How we think about supplements
  – Why do we take them?

• How we & others use supplements
  – Compared: Australia, US, Netherlands
  – What are we trying to treat?

• Current therapies and supplements
  – Supplements vs what are still in the pipeline

• Examples of some supplements
  – And how they might work
For 1 gentleman

- Prescribed:
  - Testosterone

- Self-prescribed:
  - Ubiquinol 300mg d
  - D-ribose 1-2 tsp d
  - Acetyl-carnitine 1-2 tsp d
  - “Meta B” 1 d
  - “Cell Food” 10 drops d, Glutamine 1 tsp d
    - “Tried many things, these WORK!”

= 1 medication + 6 supplements
For another gentleman

• Prescribed
  – Warfarin 5mg nocte, Mirtazapine 15mg nocte, Targin 30/15mg bd, Tramadol 50mg PRN, Paracetamol 1g tds/PRN, Dutasteride 500 mcg/ tamsulosin HCl 400 mcg) d, Candesartan 8mg m, Ezetimibe 10mg d, Tiotropium 18ug cap d, Seretide 250/50 ug inh bd, Natural tears, Zoledronic acid 5mg inf yearly, Creon 25000x2 tds, Omeprazole 20mg mane, Valium 2.5-5mg nocte; Motilium 10mg tds,

• Self prescribed
  – Policosinol 1 d, Bitter melon 1 d, CoEnzyme Q10 400mg bd, Magnesium chelate 400mg bd, Ginseng 1 d, “Digestive Enzymes”, “Liver Tonic”, Diatomaceous Earth 1 tsp

= 16 medications + 8 supplements…in 1 day
One more example

- Prescribed:
  - Keppra 500mg/1g, Luvox 250mg nocte, Motilium 10mg m, Omeprazole 20mg d, Novorapid 4 units tds ac, Lantus 13 units, Minocycline 50mg d units, Nurofen 2 caps PRN, Aspro, Bactroban 2%

- Advised
  - L-Arginine 0.75mg x7 caps qid, Taurine 1000mg x5 bd, 1000mg x 4 bd;

- Additional supplements:
  - Lysine 800mg tds, Acetyl L-Carnitine 1g, Pyrroloquinoline 20mg 4x/day , Magnesium 500mg chelate, Fish Oil 2000mg d, MultiEssentials – Ethical Nutrients 1 d, Vit D 1000 IU d, Macuvision Plus 2 d, Bilberry 12000mg 2/d, CoEnzyme Q10 150mg/Vit E 15 IU, “Executive B” 1 d, “Clear Skin” Zinc 12mg, Vit A 1500 IU 2/d; Tumeric 12.5g x 2 tabs bd, “Memory Recall”, “Mega Memory and Stress Clear” 1 d, Lacteze d, Inner Health Plus

= 7 medications, 2 advised, 20 other supplements… still in 1 day
ARTG - Labelling

- Therapeutic goods –
  Australian Register of Therapeutic Goods (ARTG)
  - can be lawfully supplied in Australia

- “AUST R” (registered) medicines
  - assessed for: safety, quality, **effectiveness**
    - all prescription-only medicines
    - over-the-counter: pain relief, coughs & colds, antiseptics

- “AUST L” (listed) medicines
  - assessed for: safety, quality, __________
    - Pre-approved low-risk ingredients.
    - E.g. sunscreens > SPF4, vitamins, minerals, herbal and homoeopathic products.
    - A **purpose** must be included on the label.
Mitochondrial disease patients' perception of dietary supplements’ use

Amel Karaa a,*,1, Joshua Kriger b, Johnston Grier b, Amy Holbert c, John L.P. Thompson b, Sumit Parikh d, Michio Hirano e

Any mitochondrial disease
  • Retrospective
  • Self-reported data
  • N = 162

Majority are/ have been on dietary supplements
  • 75% take > 4 supplements:
    • Majority reported benefits
    • Perceived benefits
      – at 2 weeks – 4 months
    • Safe

95% pay up to US$500/month

Despite other Rx:
  • Medications
  • Physical therapy
  • Diet

45.5% thinks supplements are the only intervention improving their symptoms
Most reported symptoms

- Fatigue 61%
- Subjective weakness 50%
- Temperature instability 48%
- Exercise intolerance 42.5%
- Myalgia 38%
- Irritable bowel 33%
- Ptosis 30%
- Headaches/Migraines 28%
- Anxiety 25%
### Table 2
Most frequent patients/parents reported symptoms.

<table>
<thead>
<tr>
<th>Constitutional</th>
<th>Musculoskeletal</th>
<th>Neurological</th>
<th>Gastro-Intestinal</th>
<th>Cardiac</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic fatigue</td>
<td>61%</td>
<td>Weakness 50%</td>
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<tr>
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<td>Protsis</td>
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<td>28%</td>
<td>Syndrome</td>
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<tr>
<td>Developmental delay /Intellectual disability</td>
<td>27%</td>
<td>Dysphagia 25%</td>
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</tbody>
</table>

High frequency symptoms (in >50% of patients)

Medium frequency symptoms (in 25-50% of patients)
Karaa et al 2016

• Most common supplements
  – CoEnzyme Q10 (Ubiquinol/ Ubiquinone) 42.5%
  – L-carnitine 36%
  – Riboflavin 26.5%
  – Vitamin D 24%
  – Vitamin C 15%

• 72% no side-effects

• Most common side effects
  – Nausea/ upset stomach 47%
  – Diarrhoea 17%

• < 6% patients discontinued Rx due to side effects
## Types of supplements

| Dietary supplement intake reported by patients. |
|-----------------|-----------------|-----------------|
| Supplements      | Frequency of intake (%) | Daily doses |
| l-carnitine     | 58 (36)          | 330–5000 mg     |
| Ubiquinol       | 51 (31.5)        | 30–2000 mg      |
| Vitamin B2 (riboflavin) | 43 (26.5)  | 5–400 mg        |
| Vitamin D       | 39 (24)          | 400–50,000 IU   |
| Vitamin C (ascorbic acid) | 24 (15)   | 120–1500 mg     |
| Vitamin B12 (cobalamin) | 22 (13.5) | 48–1000 mcg     |
| Alpha lipoic acid | 22 (13.5)       | 50–2400 mg      |
| Creatine        | 22 (13.5)        | 0.5–12 g        |
| Vitamin B1 (thiamin) | 21 (13)    | 5–300 mg        |
| Vitamin E (tocopherol) | 20 (12)   | 200–2000 IU     |
| Ubiquinone      | 18 (11)          | 30–2400 mg      |
| Calcium         | 16 (10)          | 200–2000 mg     |
| Vitamin B6 (pyrodoxin) | 14 (8.6)  | 5–100 mg        |
| Magnesium (bislglycinate, gluconate, citrate, rotate) | 13 (8) | 133–1200 mg |
| Vitamin B3 (niacin) | 13 (8)    | 25–550 mg       |
| Folic acid      | 12 (7)           | 0.8–800 mg      |
| l-arginine      | 10 (6)           | 0.5–18 g        |
| Other supplements use reported | | |
| Pro biotics     |                | Blue green algae |
| MCT oil         |                | Taurine         |
| Multivitamin    |                | Tocotrienols    |
| B complex       |                | Ornithine       |
| Brewers yeast   |                | Alpha-ketoglutarate |
| Fish oil        |                | Tart cherry juice |
| Idebenone       |                | Green tea extract |
| l-glutathione   |                | Garlic          |
| Methionine      |                | Carotinoids     |
| NADH            |                | Flavinoids      |
| Coconut oil     |                | Turmeric        |
| Potassium gluconate |         | Ginger          |
| SAM-e           |                | Sodium pyruvate |
| Milk thistle    |                | Spirulina       |
Karaa et al 2016

• Effective
  – 54% reported 5 most bothersome symptoms alleviated
    • Fatigue 49% improved
    • Exercise intolerance 26% improved
    • Muscle pains 26%
    • Weakness 26%

• Less responsive <12% improved
  – Gastrointestinal dysmotility symptoms
  – Neurological symptoms
    • Headache, seizures, myoclonus, spasticity

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<td>High frequency symptoms (in &gt;50% of patients)</td>
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Interesting observations

• No correlation between type of disease & response

• 62/162 thought no improvement
  – But 38% - benefit noted once off supplements

• Among patients reporting no subjective benefits
  – Only 26% discontinued use!
    • Supplements covered by insurance in only 9%
    • 26% respondents pay US$200-500 /month
• Dutch study
  – paediatric n= 24/38, adult n= 33/46 -> 57 responses
• 88% Children, 91% adults
  – uses complementary/alternative medicine
    • Children: €489/year
    • Adults: €359/year ~ $580/year
• 60% adults reported therapies to be effective
  – Food supplements, homeopathy, self-help techniques
• Vitamin supplements e.g. riboflavin, CoEnzymeQ10, Thiamine
  – not perceived to be effective
• Self-help techniques e.g. massage, yoga
  – rated positively

High prevalence of complementary and alternative medicine use in patients with genetically proven mitochondrial disorders

Sebastian Franik · Hidde H. Huidekoper · Gepke Visser · Maaike de Vries · Lonneke de Boer · Marlon Hermans-Peters · Richard Rodenburg · Chris Verhaak · Arine M. Vlieger · Jan A. M. Smeitink · Mirian C. H. Janssen · Saskia B. Wortmann
Supplements

• How we think about supplements
  – Why do we take them?

• How we & others use supplements
  – Compared: Australia, US, Netherlands
  – What are we trying to treat?

• Current therapies and supplements
  – Supplements vs what are still in the pipeline

• Examples of some supplements
  – And how they might work
Many different ways of trying to manipulate genetics
  – Difficulty introducing gene/ product
    • To various tissues
    • Blood brain barrier
    • Through the mitochondrial membrane
  – Can be risky - while cell/ animal models may show promise
    • Effects unknown in human
      – Some likely detrimental
  – Await further animal models
  – Early human trials

Small molecules
  – Aiming to boost residual mitochondrial function
<table>
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<tr>
<th>Manipulating DNA</th>
<th>Examples</th>
<th>Challenges</th>
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<tbody>
<tr>
<td>Mitochondrially-targeted nucleases</td>
<td>Zinc Finger Nucleases</td>
<td>Lack of mutation-derived restriction sites for restriction endonucleases to target</td>
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<td></td>
<td>Transcription Activator Like Effector Nucleases</td>
<td>Specificity of restriction endonuclease targeting</td>
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<tr>
<td>Manipulating mtDNA with peptide nucleic acids</td>
<td>Human non-cognate mitochondrial leucyl tRNA synthetase</td>
<td>Efficiency of targeting recombinant proteins into cells and mitochondria</td>
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<tr>
<td>Manipulating tRNA enzymes</td>
<td>Carboxy-terminal domain of human mitochondrial leucyl tRNA synthetase</td>
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<td>Gene transfer using adeno-associated viral vectors</td>
<td>AAV-ETHE1 (nuclear gene)</td>
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<td>AAV-ND4 (mitochondrial gene)</td>
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<th>New protein delivery</th>
<th>Examples</th>
<th>Challenges</th>
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<tr>
<td>Systemic protein delivery</td>
<td>Transfusion of platelets or erythrocyte encapsulated thymidine phosphorylase</td>
<td>Sustaining thymidine phosphorylase levels</td>
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<tr>
<td></td>
<td>Mitochondrially targeted transcription factor A</td>
<td>Over expression is reported to increase mtDNA copy number, mtDNA deletions and respiratory deficiency</td>
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<th>Small molecule pharmaceuticals</th>
<th>Examples</th>
<th>Challenges</th>
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<tr>
<td>Manipulating mitochondrial and nuclear DNA</td>
<td>Bezafibrate, Resveratrol, PAPR inhibitors, Rapamycin, Cyclosporin A</td>
<td>Hepatomegaly and abnormal lipid metabolism in animal models</td>
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<td></td>
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<td>Potential hepatic side effects</td>
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<td>Mild gastrointestinal side effects</td>
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<td></td>
<td></td>
<td>Mild side effects including fatigue, nausea, vomiting and anemia</td>
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<td>Hyperlipidemia, poor wound healing and immunosuppression</td>
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<td>Immunosuppression</td>
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<th>Stem cell approaches</th>
<th>Examples</th>
<th>Challenges</th>
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<tbody>
<tr>
<td>Exogenous stem cell therapy for nuclear gene mutations</td>
<td>Bone marrow and stem cell transplantation</td>
<td>Availability of stem cell transplants</td>
</tr>
<tr>
<td>A source of thymidine phosphorylase</td>
<td></td>
<td>Sustaining effect</td>
</tr>
<tr>
<td>Endogenous stem cells shifting heteroplasmy</td>
<td>Bupivacaine injections</td>
<td>Side effects of myotrophic protocol</td>
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<td></td>
<td>Exercise</td>
<td>Finite capacity of repair</td>
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<td>Limited to patients with isolated mitochondrial myopathies</td>
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</tbody>
</table>
Figure 4 Schematic representation of pharmaceutical modulators of mitochondrial biogenesis. There are multiple signalling pathways involved in mitochondrial biogenesis. PGC-1α (encoded by PPARG1A), which is a co-activator for a family of transcriptional factors known as PPARs, co-ordinates via a cascade of nuclear encoded proteins the vast majority transcriptional mitochondrial biogenesis. Novel pharmacological therapies aim to modulate PGC-1α mtDNA expression (e.g. PPARα) and protein expression or target downstream pathways. Bezasfrate is a pharmacological ligand for the transcriptional co-factor PGC-1α. AICAR activates AMP-activated protein kinase (AMPK) and is thought to modulate increased mitochondrial biogenesis through PGC-1α. The natural polyphenol resveratrol activates sirtuin 1 (SIRT1). Sirtuins are part of a group of oxidizing NAD-dependent protein deacetylases. Upon activation, for example, by PGC-1α or transcription factor A, mitochondrial (TFAM) they promote mitochondrial respiratory chain activities and the transcription of genes modulating mitochondrial biogenesis and function. Nicotinamide riboside can be used to supplement NAD+ levels. PARP1 functions as a NAD+ consuming enzyme. Thus in turn inhibition of PARP1 has been demonstrated to increase NAD+ bioavailability and SIRT1 activity (not shown above) promoting oxidative phosphorylation. Rapamycin inhibits mTOR, which in turn releases mTOR inhibition of autophagy. Cyclosporin A inhibits the mitochondrial permeability transition pore (MPTP). Opening of the mitochondrial permeability transition pore is thought to deplete pyridine nucleotides thus impairing mitochondrial oxidative respiration.
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• How we think about supplements
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• How we & others use supplements
  – Compared: Australia, US, Netherlands
  – What are we trying to treat?

• Current therapies and supplements
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• **Examples of some supplements**
  – And how they might work
New treatments for mitochondrial disease—no time to drop our standards


Table 1 | Treatments evaluated in patients with mitochondrial diseases

<table>
<thead>
<tr>
<th>Agent</th>
<th>Specific mechanism(s) of action</th>
<th>Highest level of clinical study in humans</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Increase of substrate supply to respiratory chain</strong></td>
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<tr>
<td>Carnitine</td>
<td>Fatty acid transfer for citric acid cycle intermediates</td>
<td>Case report[71]</td>
</tr>
<tr>
<td>Niacin</td>
<td>Precursor for NADH, which transfers electrons from intermediates to the respiratory chain</td>
<td>Case report[72]</td>
</tr>
<tr>
<td>Thiamine</td>
<td>Enhancement of pyruvate dehydrogenase to decarboxylate pyruvate for oxidation</td>
<td>Case report[73]</td>
</tr>
<tr>
<td>Dichloroacetate</td>
<td>Inhibition of pyruvate dehydrogenase kinase to increase availability of pyruvate for oxidation</td>
<td>Randomized, placebo-controlled crossover trial in MELAS due to m.3243A&gt;G mutation (negative outcome)[74]</td>
</tr>
<tr>
<td><strong>Augmentation of respiratory chain components</strong></td>
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<tr>
<td>Riboflavin</td>
<td>Precursor for flavin adenine dinucleotide, an electron carrier bound to complexes I and II</td>
<td>Open-label study in complex I deficiency (positive outcome)[75]</td>
</tr>
<tr>
<td>Coenzyme Q(10)</td>
<td>Electron carrier from complexes I and II to complex III</td>
<td>Randomized, placebo-controlled crossover trial (negative outcome)[76]</td>
</tr>
<tr>
<td>Idebenone</td>
<td>Analogue of coenzyme Q(10)</td>
<td>Randomized, placebo-controlled trial in Leber hereditary optic neuropathy (negative outcome)[77]</td>
</tr>
<tr>
<td>EPI-743</td>
<td>Analogue of vitamin E</td>
<td>Open-label study in Leigh syndrome and Leber hereditary optic neuropathy (positive outcome)[78,79]</td>
</tr>
<tr>
<td><strong>Bypass of respiratory chain components</strong></td>
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<tr>
<td>Succinate</td>
<td>Citric acid cycle intermediate which donates electrons directly to complexes I and II, thus partially bypassing complex I</td>
<td>Case report[80]</td>
</tr>
<tr>
<td>Vitamins C and K</td>
<td>Bypass of complex III</td>
<td>Case report[81]</td>
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<tr>
<td><strong>Energy buffering</strong></td>
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<tr>
<td>Creatine</td>
<td>ATP storage in muscles via the creatine phosphokinase system</td>
<td>Randomized, placebo-controlled crossover trials in mitochondrial myopathies (negative outcomes in two trials, positive surrogate end points in one trial)[82,83]</td>
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<tr>
<td><strong>Antioxidant activity</strong></td>
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<tr>
<td>Cysteine</td>
<td>Increases muscle availability of glutathione peroxidase</td>
<td>Randomized, placebo-controlled crossover trial in progressive external ophthalmoplegia (negative outcome)[84]</td>
</tr>
<tr>
<td>Lipoic acid</td>
<td>β-ketoacid dehydrogenase cofactor with antioxidant properties</td>
<td>Case report[85]; randomized, placebo-controlled crossover trial (with creatine and coenzyme Q(10); negative outcomes in various mitochondrial myopathies)[86]</td>
</tr>
<tr>
<td>Dimethylglyoxine</td>
<td>Antioxidant activity</td>
<td>Randomized, placebo-controlled crossover trial in Säguesay Lac-St-Jean cytochrome c oxidase deficiency (negative outcome)[87]</td>
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<tr>
<td><strong>Oxidative capacity adaptations</strong></td>
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<tr>
<td>Aerobic exercise training</td>
<td>Reversal of deconditioning and/or mitochondrial adaptation to improve oxidative capacity</td>
<td>Randomized, non-blinded controlled trial in mitochondrial myopathies (positive outcome)[88,89,90]</td>
</tr>
<tr>
<td>Resistance exercise training</td>
<td>Myofibre regeneration and presumed gene shifting</td>
<td>Open-label study (positive outcome)[91,92]</td>
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<td><strong>Nitric oxide metabolism</strong></td>
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<td>Arginine</td>
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Aiming to improve mitochondrial function

- **Enhance respiratory chain function:**
  - Thiamine
    - enhance pyruvate dehydrogenase activity
  - Nicotinamide riboside
  - Carnitine
    - Help transfer of fatty acid

- **Enhance electron transfer**
  - Riboflavin
    - Flavin adenine dinucleotide in Complex I, II
  - CoEnzyme Q10
    - From Complexes I and II to III
  - Idebenone, EPI-743
Aiming to improve mitochondrial function

- Bypass specific respiratory chain complexes
  - Vitamin C, K
  - Bypasses Complex III

- Antioxidants – ↓ toxic metabolites
  - Vitamin C, E
  - Alpha lipoic acid
  - Idebenone, CoEnzyme Q10

- Energy buffering
  - Creatine
    - Increase ATP storage through the creatine phosphokinas system
• Most commonly used:
  • CoQ10
  • L-carnitine, Creatine
  • Alpha-lipoic acid
  • B-vitamins/ Riboflavin
• CoEnzyme Q10 – should be offered to most patients
  – Ubiquinol – most bioavailable
• Alpha lipoic acid & Riboflavin
  – should be offered
• Folinic acid
  – considered in those with CNS manifestations
• L-Carnitine for patients with documented deficiency
  – Levels monitored
CoEnzyme Q10

- **Final synthesis steps in the mitochondria**
  - Involve >12 proteins in yeast, by COQ genes
- **↓ in 22-36% mitochondrial myopathies**
  - (Sacconi et al. 2010; Montero et al. 2005)
    - Improved ex tolerance, fatigue, cramps, stiffness 7/8 patients
- **Anti-Oxidant**
  - Scavenges reactive oxygen species
- **Mobile electron carrier**
  - Transports electron: Complex I & II to III
- **150mg x 2 tabs (300mg) daily**
  - ~ $2/day
Coenzyme Q

• Ubiquinone (oxidised form)
  – Lipophilic molecule, in cell membranes, mitochondria
  – Transfers electron: Complexes I & II to Complex III
  – Cofactor of dehydrogenases
  – Modulator of permeability transition pore
  – Essential antioxidant

• Ubiquinol (reduced form)
  – Better bioavailability shown in rats
CoEnzyme Q10

- **Safe**
  - No adverse effects to 3000-3600mg/d

- **Randomised, placebo-controlled, double-blind, cross-over trial**
  - N = 30
    - 15 with MELAS, 11 with CPEO
  - CoQ10 at 600mg twice a day for 60 days
  - Minor effects on
    - Cycle exercise aerobic capacity (15 mins)
    - Post-exercise lactate

- **✓Primary CoEnzyme Q10 deficiency**
  - Clinically and genetically heterogeneous disorder
    - Onset from birth to 7th decade
Idebenone

- Short-chain benzoquinone
  - Synthetic analogue of CoQ10
    - Shorter, less lipophilic tail to quinone
      - Different solubility
  - Antioxidant
  - Mitochondrial electron carrier
    - Bypasses Complex I
    - Transfers electrons to Complex III
      - Helpful in Complex I deficiency
- 150mg tabs x 60 ~ $62 ie $6.2/day
- For 900mg/d for 6 months
  - = $5766.93
• Rescue of Hereditary Optic Disease Outpatient Study – a randomised, placebo-controlled, double-blind clinical trial
  – Leber’s hereditary optic neuropathy
    • N= 85 Idebenone vs placebo 2:1 ratio
    • First vision loss within 5 years of enrolment
  – Benefitted those with discordant visual acuities
    • Benefit persisted after ~ 30 months
  – Idebenone oral 300mg x3 a day
    • for 24 weeks
      – Persistent beneficial effects in preventing further vision impairment
      – Promoting recovery of inactive-but-viable retinal ganglion cells
Idebenone

- Linear dose-proportional pharmacokinetic profile
  - Rapidly absorbed
    - Median time to max plasma concentration
      - 0.67h to 1.17h after high fat meal
  - Take with food increases bioavailability 5-7x
  - Crosses blood brain barrier
  - Rapid, extensive first-pass metabolism

- Phase 2a Clinical trial of idebenone in MELAS
  - N= 27 completed end 2015
  - Dose 900-2250/day
    - Measure of lactate – on MR spectroscopy, venous
    - Fatigue scale
  - Results still pending publication
L-arginine

• Dose oral 0.3mg/kg/d maintenance
  – If 70kg -> 21g/d = 7 capsules x3/day
    • 500, 750, 1000mg capsules, powder
      – 5-20g twice daily

• ~$4400/year

• In patients with recurrent stroke-like episodes
  – MELAS – mitochondrial encephalopathy, lactic acidosis and stroke-like episodes
L-arginine

- Nitric oxide precursor/donor
- In MELAS + stroke-like episodes
  - ✔ ↓ recovery time from episodes
  - ✔ ↓ frequency
  - ✔ ↓ severity
  - N= 24; 34 stroke-like episodes
    » Koga et al. 2005)

• Loading dose of L-arginine 0.5g/kg given within 3 h of symptom onset
  – IV infusion for next 3-5 days
    • “though no clinical evidence on how long to continue”
• Dextrose containing fluids
  – To avoid catabolism
• Oral L-arginine 0.15-0.30g/kg
  – 3 divided doses to continue
L-Arginine Affects Aerobic Capacity and Muscle Metabolism in MELAS (Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-Like Episodes) Syndrome

Lance H. Rodan¹, Greg D. Wells²,³, Laura Banks²,³, Sara Thompson³, Jane E. Schneiderman²,³, Ingrid Tein¹,⁴*

• Perhaps improves aerobic capacity/muscle metabolism in MELAS/m.3243A>G mutation
  – N = 3 MELAS young siblings, 4 controls
    • Only MELAS patients had L-arginine
  – Single dose
  – 6 week supplementation at 0.1g/kg/d
Impaired nitric oxide production in children with MELAS syndrome and the effect of arginine and citrulline supplementation

Ayman W. El-Hattab a, b, Lisa T. Emrick e, Jean W. Hsu d, Sirisak Chanprasert a, c, Mohammed Almannai a, c, William J. Craigen a, c, Farook Jahoor d, Fernando Scaglia a, c, *

- **Oral L-Citrulline -> L-Arginine in kidneys**
- **N = 5 children with MELAS, 5 controls**
  - Nitric oxide production rate increased
    - Higher with Citrulline compared to Arginine
  - Citrulline supplementation increased de novo arginine synthesis
Taurine

- Role in modifying genetic code
  - Post-transcriptional modification in tRNAs
  - Defective in mutant mitochondrial tRNA\(^\text{Leu}\)
- In cell model
  - Taurine ameliorates impaired mitochondrial function
- Prevented stroke-like episodes in 2 MELAS patients for > 9 years
  - 21 yo male, 29 yo female
  - High dose 0.25g/kg/d
- If 70kg -> 17.5g/d
  - 1g capsules – 100 cap = $15
    - Lasts 5 days
Creatine

- Creatine monohydrate 5g bd x14d -> 2g bd x 7d  
  (Tarnopolsky et al, 1997)
  - N = 7 (lactate > 2.7mmol/L at rest, severe ex intolerance, RRF, most multisystemic)
  - Randomised controlled trial
    - ADL, handgrip for 1 min, exercise lactate… aerobic cycle ergometry
    - Increased handgrip strength, post ex lactate, strength of high intensity activities, but **not for lower intensity aerobic activities**

- Creatine monohydrate 20g/d x 4 weeks  
  (Klopstock T, et al, 2000)
  - N = 16 CPEO + MM patients
  - **Well tolerated, but no significant effect** on exercise, eye movements, ADL
L-Carnitine (Levocarnitine)

- (Gimenes et al, 2015)
  - N = 12 CPEO+MM, 10 controls
    - L-carnitine supplementation 3g/d vs placebo
  - Clinical status, body composition, respiratory function, muscle strength, cardiopulmonary ex test after 2 months
    - May improve aerobic capacity, ex tolerance during high intensity work

- L-carnitine 1g daily in am, to 3x/day
  - For adolescents and adults


- **Reduce oxidative stress**
  - Control by scavenging reactive oxygen species

*Teixeira et al, 2017*
The Sirtuin pathway

• **Resveratrol (in wine)**
  – Mimics anti-ageing effects of caloric restriction
  – Increases SIRT1
    • Helps mice on high fat diet from diabetes, weight gain

• **Pterostilbene**
  – More bioavailable than resveratrol in mice

• **NR (small amount in milk) -> NAD+**
  – Fuels sirtuins activity
    • SIRT 1 helps insure signal between cell’s nuclei and mitochondria
    • SIRT 3 keeps mitochondrial running well
Resveratrol

- Natural polyphenol
  - From red wine, skin of grapes, blueberries, raspberries
- Increases NAD+ (oxidized nicotinamide adenine dinucleotide)
  - Activate protein deacetylase SIRT1
    - Activates PGC-1alpha
      - Promoting mitochondrial biogenesis and function
        » Canto & Auwerx, 2009; Kanabus et al., 2014
- Showing promise in Friedreich’s ataxia
  - Open-label pilot clinical study
    - Mild GI side effect
      » Delatycki, 2012; Yiu et al., 2013
- Restore normal function in human fibroblasts with problem with mitochondrial fatty acid beta-oxidation
  » Bastin et al., 2011; Mizuguchi et al, 2017
- Decrease oxidative stress in case of mitochondrial complex I deficiency
  - Restore O2 consumption in complex I deficient patient cell lines
- Cost
  - 1 cap = 420mg trans-resveratrol (70mg grape seed extract)
  - 60 caps = AU$38
Nicotinamide riboside

- Aim to increase NAD+ bioavailability - precursor
  - Form of Vitamin B3
  - To improve oxidative phosphorylation
    - Advertised for “endurance, performance, weight management, cardiovascular health, anti-aging, cognitive function, neuroprotection”

- Promising in
  - Mouse model – Sco2^{KOKI} (nuclear gene) mice
    - Improved ex intolerance
      - Cerutti et al., 2014
  - Deletor mouse (nuclear gene mutation causing mtDNA deletions)
    - Improve mitochondrial biosynthesis
      - Khan et al., 2014
      - Dose 400mg/kg/d

- 100-125mg tabs
  - 60 tabs = AU$52.48
NR, NAD, NMN

• **Mouse heart failure model** (Lee et al, 2016)
  - NMN (nicotinamide mononucleotide)
    - Rebalanced NADH/NAD+ to improve hyperacetylation, help heart failure
  - -> **Clinical trial of human Nicotinamide Riboside 2g/d for 12 weeks**

• **CPEO + MM patients** (Pirinen et al, 2017 abstract)
  - Niacin (Vit B3)
  - Niacin improved ex capacity in CPEO
    - 6MWT improved after 1 mo
    - Improved muscle strength especially back, UL.
    - Increased HDL, but not changed HDL fraction.
      - Reduced sig apolipoprotein B1, and relieved muscle pathology
        » But side effects significant of flushing…
  - -> Investigator is now trying a study on obesity -> nicotinamide riboside 1g e.g. 500mg bd
Riboflavin

• Riboflavin 100-300mg/d
  • Water-soluble Vitamin B2
  • Precursor of:
    – Flavin mononucleotide (FMN) – co-factor for NADH-CoQ reductase in complex I,
    – Flavin adenine dinucleotide (FAD) – complex II activity as electron carrier and cofactor for succinate dehydrogenase
  • Enhances complex I and IV in C. elegans (Grad, et al 2006)
    – Complex 1 deficiency
      • Riboflavin at high dose effective
    – Case reports N <5
Folinic Acid

- Folate, Folic acid, Folinic acid
  - dihydrofolate reductase
- Kearns-Sayre Syndrome
  - Cerebral folate deficiency
- N = 6
  - Aged 8-17 years
  - 1-3mg/kg/d
- Outcome
  - MRI
  - Cerebral 5-methyltetrahydrofolate deficiencies
  - Newcastle paediatric mitochondrial disease scale
- 800ug cap
  - 60 cap for $15
    - For a 40 kg person at 1mg/d
    - Need to take 50 caps a day!!
Supplements

• How we think about supplements
  – Why do we take them?

• How we & others use supplements
  – Compared: Australia, US, Netherlands
  – What are we trying to treat?

• Current therapies and supplements
  – Supplements vs what are still in the pipeline

• Examples of some supplements
  – And how they might work
Home message

• Human clinical trials ongoing
  – Larger, newer agents

• Cell & animal studies not always translate into human use
  • Animal studies - 1/3 -> Human trials – 1/10 to use

• N of 1 trial
  – Don’t keep accumulating
    • If without clear effect
    • Use $$$ wisely

Pfeffer, et al. Nat Rev Neuro 2013
Clinical trials

- Arginine and Citrulline on endothelial dysfunction
- EPI-743
- Idebenone (Raxone)
- Bezafibrate
- Elamipretide
- KHENERGY
<table>
<thead>
<tr>
<th>Row</th>
<th>Saved</th>
<th>Status</th>
<th>Study Title</th>
<th>Conditions</th>
<th>Interventions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>Recruiting</td>
<td>Short-term Outcome of N-Carbamy glutamate in the Treatment of Acute Hyperammonemia</td>
<td>Propionic Acidemia, Type I and/or Type II, Methylmalonic Acidemia, Carbamoyl-Phosphate Synthase I Deficiency Disease, Ornithine Carbamoyltransferase Deficiency</td>
<td>Drug: Carbaglu, Drug: Placebo, Drug: Standard Care Treatment</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Active, not recruiting</td>
<td>Efficacy Study of GS010 for the Treatment of Vision Loss up to 5 Months From Onset in LHON Due to the ND4 Mutation</td>
<td>Optic, Atrophy, Hereditary, Leber</td>
<td>Genetic: GS010, Other: Sham Intravitreal Injection</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Active, not recruiting</td>
<td>Rehabilitative Trial With Cerebello-Spinal tDCS in Neuodegenerative Ataxia</td>
<td>Ataxia, Cerebellar: Cerebellar Ataxia, Spinocerebellar Ataxias, Ataxia, Spinocerebellar, Spinocerebellar Ataxia Type 1, Spinocerebellar Ataxia Type 2, Spinocerebellar Ataxia 3, Spinocerebellar Degenerations, Friedreich Ataxia, Ataxia With Oculomotor Apraxia, Multiple System Atrophy</td>
<td>Device: Anodal cerebellar and cathodal spinal tDCS, Device: Sham cerebellar and sham spinal tDCS</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Active, not recruiting</td>
<td>Efficacy Study of GS010 for Treatment of Vision Loss From 7 Months to 1 Year From Onset in LHON Due to the ND4 Mutation</td>
<td>Optic, Atrophy, Hereditary, Leber</td>
<td>Genetic: GS010, Other: Sham Intravitreal Injection</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Recruiting</td>
<td>Rosuvastatin (Crestor) in Friedreich Ataxia</td>
<td>Friedreich Ataxia</td>
<td>Drug: Rosuvastatin</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>Active, not recruiting</td>
<td>Safety Evaluation of Gene Therapy in Leber Hereditary Optic Neuropathy (LHON) Patients</td>
<td>Leber Hereditary Optic Neuropathy</td>
<td>Genetic: GS010</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>Recruiting</td>
<td>Exercise Study Testing Enhanced Energistics of Muscle Mitochondria in CKD</td>
<td>Moderate-severe Chronic Kidney Disease Not Treated With Dialysis, Non-Insulin Dependent Diabetic Kidney Disease</td>
<td>Behavioral: Combined Aerobic and Resistance Exercise</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>Recruiting</td>
<td>Study to Assess the Efficacy and Safety of Raxone in LHON Patients</td>
<td>Leber's Hereditary Optic Neuropathy (LHON)</td>
<td>Drug: Idebenone</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>Recruiting</td>
<td>Trial of Oral Glutamine on Mitochondrial Function in CKD</td>
<td>Cardiovascular Disease, Sarcopenia, Endothelial Dysfunction, Muscle Mitochondrial Function, Kidney Disease</td>
<td>Dietary Supplement: L-glutamine, Dietary Supplement: Maltodextrin</td>
</tr>
</tbody>
</table>

Your search: (treatment trial | Recruiting, Active, not recruiting Studies | mitochondrial disease | Adult) found only a few studies.
Putting supplements into a bigger context

• Symptomatic management
  – Medical
    • Heart
    • Lungs
    • Eye
    • Hearing
    • Seizures
    • Gastrointestinal
    • Diabetes
    • Kidney and liver function
  – Psychological support

• Allied health
  • Physiotherapy
  • Occupational therapy
  • Speech pathology
  • Dietitian
  • Orthotics

• Genetic counselling
• Graded exercises
• Supplements
• …Clinical trials
• …Future gene therapy
Thank you for your attention

• Thank you for your patience