Exogenous intervention in mRNA transcription

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Subtitle: "I ate a *lot* of citrate"

References and further info

See Website
https://citrate.science/2024poster/poster.html

Abstract

In an N=1 biohacking experiment without randomisation or placebo control, in a white man in his 60s, the use of a combination of daily citrate supplementation (varying numbers of grams between 2 and 70 per day) and histone deacetylase inhibition was found to reduce the background level of C Reactive Protein consistent with a reduction in the burden of senescent cells, encourage new hair growth and the repigmentation of hair on a dose dependent basis, enable more rapid repair of a venepuncture insult, enable easier muscle growth and improve metabolic balance. This was consistent with improving the transcription of mRNA so that the longer genes which often have difficulty being transcribed during aging were transcribed. Other individuals have experimented with a similar protocol with anecdotal reports of similar improvements and further research is warranted.

Background

Eusocial Hymenoptera (Queen Bee/Ant)
It is well known that The Queens of Bees and Ants have a different phenotype. This is material because they also live considerably longer than worker Bees which otherwise have the same genes. This has been studied in some detail in bees and it is known that the substance Royal Jelly which is eaten by Queen Bees for all of their life (but by worker bees for only 3 days) has the effect of changing the phenotype to a queen. Royal Jelly contains a weak HDAC inhibitor, a Coenzyme A precursor (Vitamin B5) and AMPK activators. Hence it would be expected to enhance mRNA transcription. Queen Termites also have a changed phenotype, but the literature is not as clear as to the reasons for this.
The use of Drosophila melanogaster as a screening agent for longevity factors; pantothenic acid as a longevity factor in royal jelly.

Royal Jelly for Homo Sapiens and XPRIZE Healthspan
In essence, therefore, a mixture of citrate, various natural HDAC inhibitors and other supplements are being used as an equivalent of Royal Jelly, but for Human Beings. This is the basis for a protocol for the "Biohacking to Improve Everyone's Health" team which has been entered for XPRIZE Healthspan.

Splicing variations and Acetyl-Coa dynamics
The flow of acetyl-CoA in the cytosol and nucleus is quite complex. The main two sources are from citrate conversion with ACLY and acetate conversion with ACSS2. However, acetyl-CoA can then be used for a number of purposes of which only one is acetylation of the histone. However, from a signalling perspective it can be seen that levels of acetyl-CoA are indicative of the power (measured in Watts) available from the mitochondria. Hence if acetyl-CoA levels are higher then genes which require more power can be produced. The balance in energy usage between transcription and translation can be controlled, therefore, by the power availability in the cell. The balance between splicing variations for any one gene will be driven at least in part by the level of power available in the cell. If a gene is not transcribed in whatever splicing format then it will not call on ATP resources for translation.

Questions about mRNA transcription
mRNA transcription has had considerable attention over the years. It is accepted that for the process of transcription to occur that the histone requires acetylation. This requires the Histone Acetylation Transferase that travels with the RNA Polymerase II complex to have acetyl-CoA as a substrate. More recently, research has pointed to difficulties with RNA Pol II being stalled. Research has also pointed to Histone Deacetylases impacting on transcription. The author's search of the literature has not identified any conclusive explanation as to the mechanism for this. The author wonders whether it may involve the deacetylation of the histone close to the point at which RNA Pol II is stalled. This could involve deacetylation of the histone just prior to where RNA Poll II is stalled so if RNA Pol II goes into reverse it encounters a termination position. Suffice to say that there is evidence that some hyperacetylation leads to ATP depletion and atoptosis. There is also considerable evidence that HDAC inhibitors of whatever class do impact on transcription as do HDAC's themselves. From a practical perspective, therefore, there is a good mechanistic hypothesis for making use of HDAC inhibitors as part of a package of interventions to interfere, hopefully in a positive manner, with transcription.

Natural HDAC inhibitors
There are a number of well known herbal substances that have been used medically for many years. A quarter involving curcumin, quercetin, pterostilbene and berberine were selected as the first intervention quartet. However, other HDAC inhibitors have been tested as well although none of the results of those experiments are reported here. The first quartet were selected because as natural compounds that have been used for a long period of time their safety record is clear. They tend to operate with an IC50 in the 10s of micromolar range.

Role of histone acetylation in gastric cancer: implications of dietetic compounds and clinical perspectives Mitochondrial Heteroplasmy, the MMP, AMPK and Traditional Chinese Medicine
The Mitochondrial Membrane Potential and rate of citrate efflux appear to be quite closely linked. This is clearly from an evolutionary perspective the signalling mechanism that acetylation is relying on. Hence as mtDNA gets damaged and heteroplasmy increases with often associated damage to the electron transport chain then the level of citrate efflux reduces. Obviously another mechanism for improving this (and probably a better one, but harder to do) is to improve the mitochondrial quality. This is probably the primary pathway through which damage causes an aging effect whereas the secondary is through the accumulation of senescence. There are a number of tools including mitophagy, for which the use of Rapamycin is helpful and other similar mechanisms. Also activating AMPK is useful. Within TCM there is a category of herbs known as Yang Qi activators. Looking at the literature these seem to be almost entirely AMPK activators. As part of the protocol some experimentation has been done with these without any conclusive result as yet although this will form part of the XPRIZE Healthspan protocol.

Citrate Flows - The ins and outs of citrate
There is clearly a question as to what extent citrate gets into cells other than liver cells. Apart from the citrate carrier that takes citrate from the mitochondria there are a number of carriers in the cell membrane itself. The main three are SLC13A2, SLC13A3 and SLC13A5 (also known as INDY in Drosophila or mINDY in Homo Sapiens). These vary in their expression in different cells, but a small amount of expression is seen in a number of cells. Additionally there is a splicing variant of the mitochondrial citrate carrier that places the protein in the cell wall rather than the inner mitochondrial membrane. This has the interesting potential effect that as acetyl-CoA levels go down in the cytosol a protein is expressed that extracts citrate from serum, if it is there. SLC13A5 is strongly expressed in liver cells and acts to draw citrate from serum. This gives rise to citrate's 30 minute serum half life. However, there is evidence for a low level of citrate transport in other tissues.

Citrate and Cancer
There have been suggestions that citrate could be used to treat cancer. There have been a number of reviews written about this and also higher serum citrate is not always a sign of better health, sometimes it is a sign of worse health. The references for this are on the citrate.science website.

Some senescent cells are those stuck in the process of differentiation
The author is working on a model of senescence where some senescent cells are stem cells which have failed to differentiate properly as a result of an underexpression of SLC25A1. Interleukin-10 is an inhibitor of NF kappa B which is a transcription factor for SLC25A1. IL-10 is also part of SASP. Hence, it is a good candidate for a feedback loop. Interestingly, there is some evidence that points to this and that IL-10 can cause senescence.

Prostate Cancer and BPH
The prostate is an interesting organ from the perspective of epigenetics in that it acts to place citrate in the seminal fluid. That has the evolutionary advantage of making it possible for the zygote to take some citrate from the external environment to assist with acetylation of the histone at the point at which rapid division is occurring.

Prostate cancer is also associated with serum citrate levels in that lower serum citrate levels point to a prostate cancer diagnosis.

Prostate cancer itself is strongly associated with aberrant splicing. It is, therefore, possible that exogenous citrate could reduce the amount of aberrant splicing. The only test results are the usual N=1 test results from one individual, but that does give a value for PSA which in most cases are below 1 microgram per litre. This varies negatively with citrate supplementation levels.

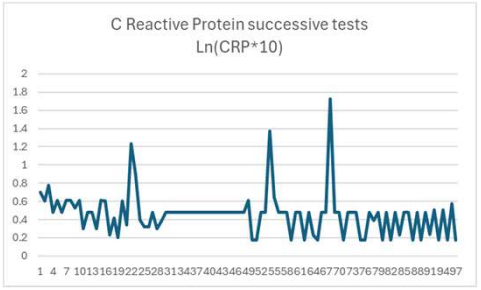
Muscle Growth and balance
The test subject improved his score on the sitting rising test from 3 to 1 (A lower score is better). He also managed to do chinups (which he has not previously been able to do).

C Reactive Protein (CRP)

CRP is a protein produced by the liver from Interleukin-6. Interleukin-6 is a cytokine both produced when an infection occurs, but also as part of the Senescence Associated Secretory Phenotype (SASP). By taking weekly CRP tests, it was possible to identify a background CRP level which when there is no infection, indicates the burden of senescent cells. This was reduced during the citrate supplementation from 0.5 mg/L to under 0.15mg/L (which is the testing threshold of the most sensitive lab test). This implies an association of citrate supplementation with a reduction in senescence. (see chart).

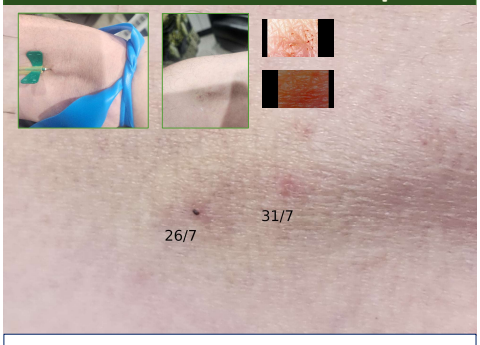
In the chart it can be seen how an infection from time to time increases IL-6 and CRP, but then it goes back down to the "too low to measure" value.

It should be noted that 0.15mg/L is a very low value for CRP for an older human male.



Notes on the chart: The chart uses the natural logarithm of the value of CRP in milligrams per litre multiplied by 10 for successive measurements of CRP. The natural logarithm is used because the high values are many multiples of the low values. One value was excluded because it appears the lab tested someone else's sample. The value is multiplied by 10 because it otherwise would provide a negative logarithm. The minimum values dominate at 0.3 and 0.15 for two labs, but the principle of infection driving the value high and then it falling back is clear.

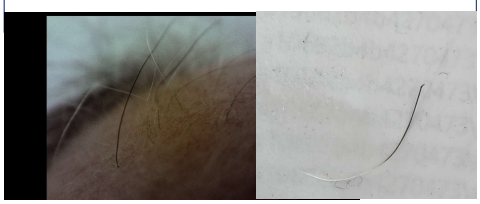
Accelerated Wound Repair



The experimenter does weekly blood tests from the same vein. The main photograph above is of the partially repaired venepuncture from 13.40 on 26/7 (no citrate) and that from 10.25 on 31/7 (15g citrate). The photograph is on 1/8 at 6.35am. This demonstrates that systemic citrate accelerates wound healing. More information on web page.

Hair (re) Growth

Citrate supplementation caused the regrowth of hair and a very very slow advancing of the hairline where it was previously receding. This was dose dependent and has resulted in vellus hairs becoming terminal and a few white hairs becoming pigmented.



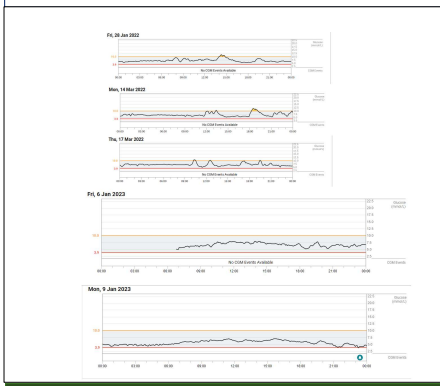
Hair regrowth and a facial hair becoming pigmented.

Phenotypic Changes

A large number of subjective phenotypic changes have occurred. These are not only as a result of citrate supplementation, but do arise from the wider protocol of which citrate supplementation is an important part.

Glucose Handling

Acetylation is required for the transcription of non-coding DNA as well as coding DNA. The results from CGM were used both prior to starting the citrate protocol as well as having been doing it for a year. This pointed to an improvement in glucose handling such that the peak following a standardised breakfast (beans, bacon, sausage, toast and tea) reduced from 10mmol/L to 8mmol/L. This pointed to glucose handling being improved such that the polyol pathway was not activated. The test subjects HbA1c remained below 5% (The minimum was 4.18%).



Conclusions

The big test for research is whether it is reproducible. That applies whether randomization or placebo are used and whatever the number of participants are in the experiment. Anecdotal reports from other users of citrate supplementation are that similar effects occur, but further *perhaps more rigorous* research is needed.

Methods
A mixture of citrate salts with a range of cations was used (Na/K/Mg/Ca). The quantity of citrate supplemented on a daily basis ranged between 2g and 70g. Because the half life of citrate in serum is around 30 minutes supplementation was normally spread out during the day in order to maintain a higher serum citrate level than is the normal level rather than a high peak that simply decays rapidly. A large range of other interventions was used, but as part of the primary intervention four HDAC inhibitors, curcumin, pterostilbene, quercetin and berberine were used. Additionally Coenzyme A precursor Vitamin B5 and vitamins B9 and B12 were supplemented. These, of course, are not the only inputs. However, they are the inputs directly relevant to mRNA transcription.

Blood was drawn weekly and tested with a range of different laboratories (primarily Randox, Medichecks, NWP, MPS, London Medical). There is some variation between different analyzers and by using a number of laboratories it becomes possible to identify patterns of variation caused by the labs rather than the underlying biomarkers varying.

Debugging the metabolism
The experimental technique of using a large number of interventions and varying them either individually or jointly and measuring any changes has been used. This is the technique used normally for debugging a computer system. It enables a complex system with a number of inputs to be monitored. Although the process is not placebo controlled or randomised the conclusions give an opportunity for other researchers to test replicability. It is, however, recommended that anyone trying this links with the author as the issue of cation clearance is complex. It is possible to have a high level of cations with good kidney biomarkers, but not that straightforward.

Renal issues
The biggest challenge with citrate supplementation is the renal clearance of cations. As citrate supplementation is increased with the citrate anion there is a need to also supplement with additional cations. The effect of this on renal markers has been noted. The optimal results for creatinine were as low as 62 micromoles, but it has gone as high as 123. Some of the higher value, however, arose from testing delay causing the sample to metabolise. With Cystatin-C the range is between 0.67 and 1.2 micrograms per litre. It is important for anyone supplementing with citrate in any substantial amounts (over 5g per day) to monitor renal markers and manage overall kidney demand (also from diet, not just supplementation).

Other points of concern
Any experimentation with Citrate at a high level in human beings should be done with care. The increase in cytosolic acetyl-CoA results in a potential increase in cytosolic ROS. This could be useful for dealing with localised infections, but if it is done rapidly in a body with many infections that were previously ignored by the immune system then there could be an increase in localised inflammation. Hence any introduction of citrate supplementation should be done with doses increased gradually. The author also supplements with melatonin which he takes to reduce the impact of ROS and RNS. Clearly also ATP limits will have an effect and increasing transcription rates can go too far.

Conflicts of interest
The author has made patent applications in connection with his discoveries.

About the author
Born 1960
Educated: King Edwards School, Birmingham.
Scholarship to Magdalen College (Oxford), MA (Oxon) in Physics.
Formed his first business in 1983 which was sold in 2019
Councillor on Birmingham City Council 1990-2008
Deputy Leader of Birmingham City Council 2004-2005
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Has been drummer in a heavy metal and punk band in the 1970s then moved into prog rock as a drummer, but more recently is the keyboard player in *"John Hemming and the Jazz Lobbyists"* which is a well known participant in the Birmingham Jazz Festival.
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