



Unlocking the secrets of Anti-aging

3-carboxy-3-oxopropanoic acid and the NAD⁺/NADH ratio

3-carboxy-3-oxopropanoic acid (3C3-OXO) mimics calorie restriction. It focuses on increasing and decreasing the activity of genes in a similar manner that calorie restriction changes the activity of the genes. Over 350 genes are expressed in a manner similar to calorie restriction, resulting in an astonishing increase in average and maximal lifespan, weight reduction, and glucose reduction in multi-species animal tests. Human clinical trials have confirmed both reduction in glucose levels and improved uptake in glucose without negative side effects. This simple modification to metabolism has major implications for cancer prevention and treatment, diabetes prevention and management, atherosclerosis, macular degeneration, and neurological decay including Alzheimer's and Parkinson's.

Benefits of modifying cellular metabolism to produce calorie-restricted conditions.

The benefits of calorie restriction have been studied for over 70 years. Calorie Restriction (CR) is the reduction in the overall amount of calories (by 30 to 50%) while maintaining proper nutrition. It has been the only proven method to extend the maximal lifespan of mammals. CR has been shown to cause major beneficial shifts in health and metabolism on a wide range of organisms, from the single cell to very complex (including humans). The wide range of success of CR indicates that the process of life extension is based on similar effects observed between species, and occurs on the molecular level of individual cells.

Mice that receive adequate nutrition but a reduction in calories have delays in the onset of many age related diseases including cancer, diabetes, and Alzheimer's [Hursting, 2003]. A reduced calorie diet also leads to an increase in average and maximal lifespan. This data strongly shows that humans will also live longer [Fontana, 2004 Ingram, 2006].

The benefits of calorie restriction are due to changes within each cell that forms the gene expression and include those involved in:

- immune response
- protein turnover
- protein synthesis

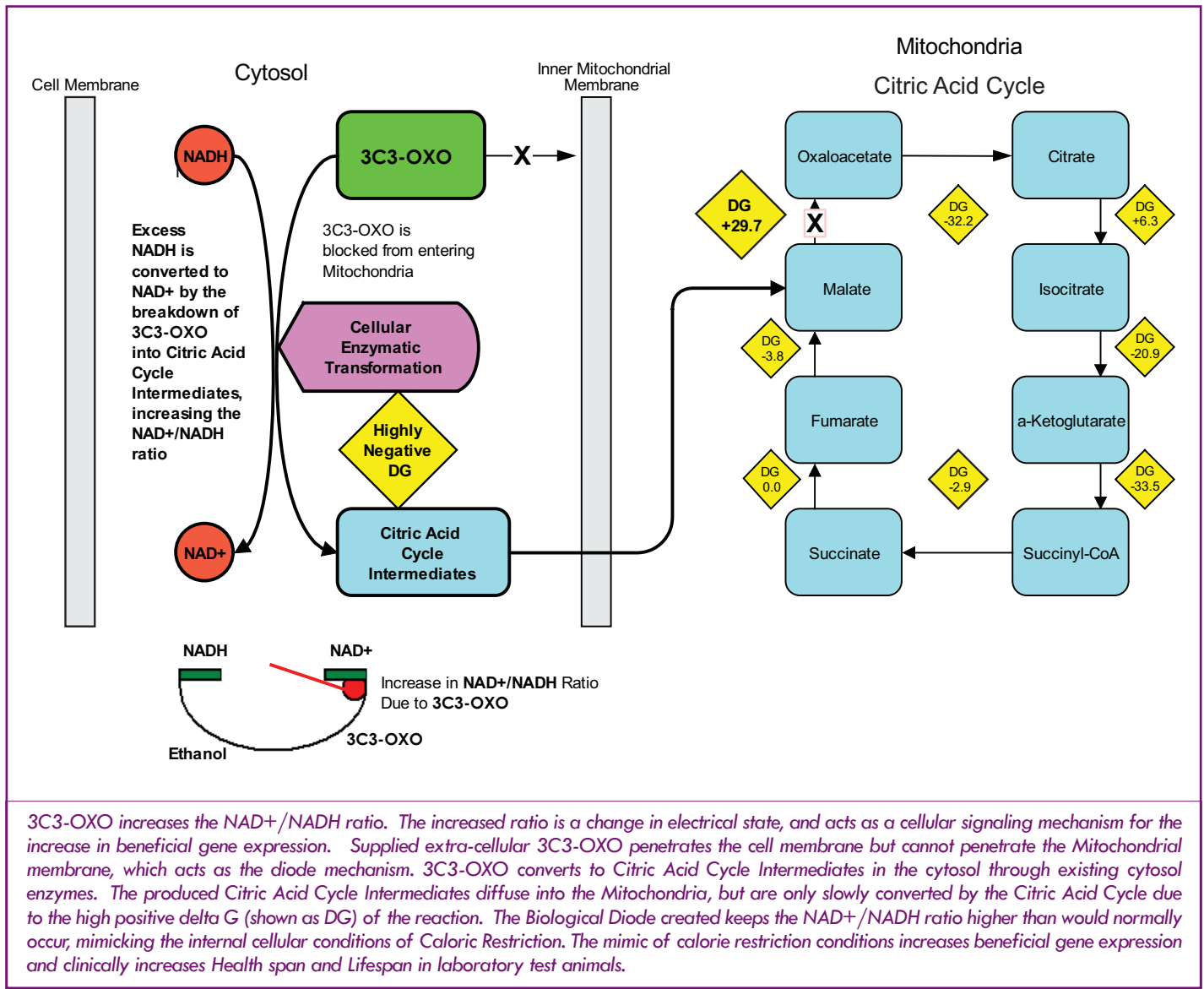
[Lee, 2004]. Masternak et. al. [2004] show that genes related to insulin and insulin growth factor 1 (IGF1) are altered including PPAR- α , a gene that is suggested to play an important role in metabolic control, accumulation and preservation of fat storage cells.

The activity of FOXO genes have also been shown to change under caloric restriction [Furuyama 2002, Daitoku, 2004]. "FOXO factors may act as tumor suppressor genes and it is the loss of their function that may be the pivotal event in tumorigenesis" [Arden, 2006, Greer 2005]. "These same FOXO genes may play a role in preventing DNA damage by inducing expression of genes important in the detoxification of reactive oxygen species (ROS)" [Arden, 2006]. Studies of humans undergoing CR for 3 to 15 years have shown reduced risk for atherosclerosis along with reductions in fasting glucose, fasting insulin, Hs-CRP levels, systolic and diastolic blood pressure, triglycerides, total cholesterol, and LDL cholesterol as compared to equivalent age-matched controls [Ingram, 2006].

How does 3C3-OXO work?

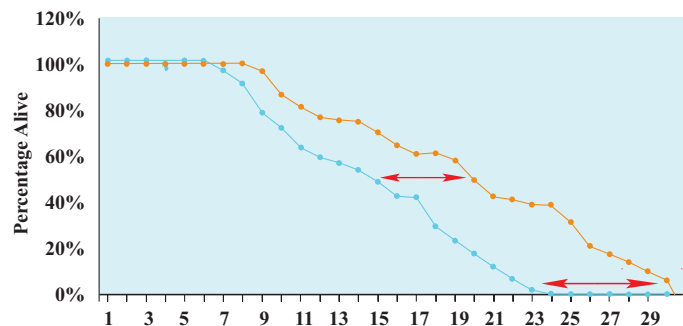
3C3-OXO mimics what happens during calorie restriction, and modifies the cellular energy pathways to activate multiple genes already shown to be beneficial. The key mechanism of action of 3C3-OXO is changing the ratio of Nicotinamide Adenine Dinucleotide (NAD⁺) and the reduced version, NADH. 3C3-OXO increases the NAD⁺ to NADH ratio by the action of a biological diode. When added to the cell, 3C3-OXO converts NADH into NAD⁺ in the cytosol and ends as a simple member of the citric acid cycle family of compounds. The inner mitochondrial membrane acts as the diode mechanism for the reaction, not allowing 3C3-OXO into the mitochondria, but forcing the reaction converting NADH into NAD⁺ in the cytosol. This changes the electrical state in the cytosol. The increased NAD⁺/NADH ratio/change in electrical state is the same critical signaling mechanism as seen in calorie restriction [Lin 2004], and results in the increased expression of many beneficial genes. These beneficial genes allow an increase in average and maximal lifespan, increased overall health, reduction in blood glucose levels and reductions in weight in laboratory animals. Clinical trials in humans have documented the reduction in blood glucose levels, but there is not sufficient information at this time to show if there will be an increase in maximal human lifespan-we should have this data in about 130 years. In the meantime, we look at the successful increases in lifespan of multiple short lived species to document the possible effect of 3C3-OXO on humans.

The effects of 3C3-OXO as a biological diode and the citric acid cycle



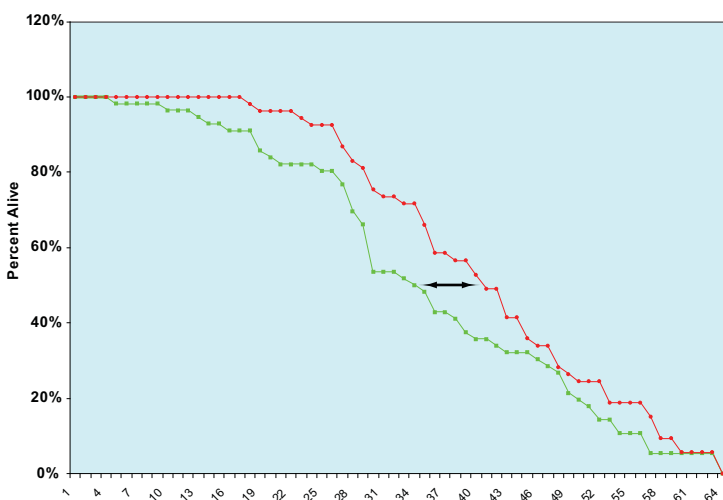
3C3-OXO increases average and maximal lifespan in multiple species by up to 40%

Investigation on biological diode compounds, and 3C3-OXO in particular, initially started on a well known worm, *Caenorhabditis elegans*. This worm is selected for many studies because its genetics are rather well understood. In adding 3C3-OXO to the agar on which the worms live, researchers observed a dose dependant increase in lifespan. The more 3C3-OXO added, the longer the life of the worms. This was an important test, as one of the major properties of the gene changes seen in calorie restricted animals is the ability to increase lifespan. Once worms reach adulthood, their cells do not divide - so the only way for them to live longer is on a cellular level, allowing each cell to live longer.



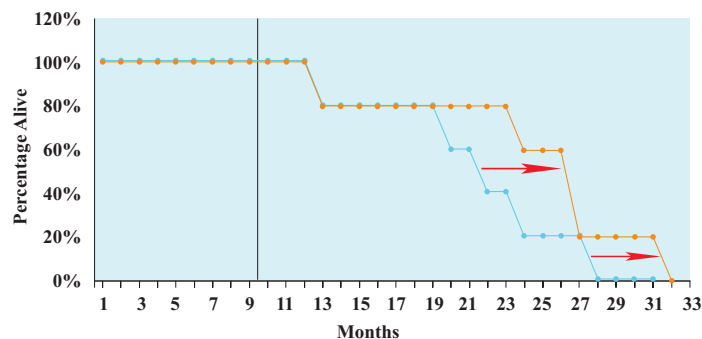
C. elegans (worms) live 36% longer than the control group when 3C3-OXO is added to the agar.

After testing the compound on worms, research was moved on to the fruit fly, which is a more complicated animal. Again, 3C3-OXO supplementation in the fly food increased lifespan. It was interesting to note that in flies, an increase in maximal lifespan was seen when the flies were placed under stress. The maximal lifespan was increased by over 100% as compared to the control group. This is primarily because stress killed off the control flies, and did not greatly affect the 3C3-OXO flies.



Fruit flies live 20% longer than the control group when 3C3-OXO is added to their food.

Worms and flies are great, but what about complex animals such as mammals? We share 98% of our DNA with mice, and the metabolic pathways are particularly similar from mice to humans. A common breed of laboratory mice, C57BL/6, was selected for the longevity experiments, and started with older males. Males were used because they typically live shorter lifespans than females. Again, in pilot testing, an increase in average and maximal lifespan was seen when 3C3-OXO was added to the mouse food. Even though the 3C3-OXO supplement was started 1/3 of the way through their lifespan, the mice still lived 25% longer than the control group. Both groups were allowed to eat unrestricted amounts of food. Maximal lifespan was increased by 14%. Not only was lifespan increased, but health span was also increased. The mice on 3C3-OXO showed less signs of inflammation, reduced incidence of spine curvature, and reduction in hair graying. 3C3-OXO is now being tested on a larger group of mice.



Supplementing mice with 3C3-OXO lead to an increase of 23% in average lifespan, and an increase in maximal lifespan of 14%. Mice were started on 3C3-OXO in their 9th month. The increase in average "Residual" lifespan was 39%, similar to what can be achieved with calorie restriction.

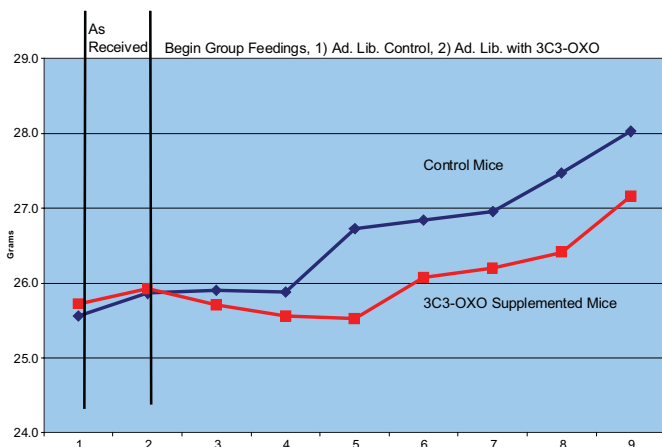
3C3-OXO interacts with genes to reduce fat content

Adding 3C3-OXO to the food of mice decreased the weight gain of C57BL/6 mice allowed to eat unrestricted amounts of food, as compared to the control group. The genes that control the storage and mobilization of fat tissue were altered reducing the overall weight gain. The effect took approximately 3 weeks to be measurable. Reduction in fat content is a positive health benefit also seen in calorie restricted mice.

3C3-OXO lowers blood glucose levels and improves glucose uptake

In a clinical trial, diabetic patients ranging from 15 to 95 years old were given 3C3-OXO for 30 to 45 days. Fasting levels of glucose in both type 1 and type 2 diabetic patients were decreased by an average of 23.7% in the trial. No negative side effects were noted. Glucose uptake by tissues was also increased in diabetic patients by 299%. Glucose uptake in non-diabetic patients was improved by 180%. The reduction of glucose in the bloodstream ties very well to a calorie restricted state. Interestingly, Kitamura [2005] showed that FOXO1 (one of the genes upregulated by both calorie restriction and 3C3-OXO) protects against pancreatic beta cell failure, which again ties to the genomic response of both calorie restriction and 3C3-OXO supplementation from the animal models. In addition, Nyengaard [2004] stated that the free NADH in the cytosol "accelerates the onset and progression of diabetic retinopathy (and other complications of diabetes)". 3C3-OXO specifically targets cytosolic NADH and converts it into NAD⁺.

It is also important to note that lower glucose levels will automatically lead to lower levels of Advanced Glycation Endproducts (AGEs), which may also lead to longer lifespan.



Adding 3C3-OXO to the food of mice decreased the weight gain of C57BL/6 mice allowed to eat unrestricted amounts of food, as compared to the control group.

3C3-OXO mimics the genomic profile of calorie restricted animals

Researchers examined the genomic profile of the mice fed the 3C3-OXO supplement as compared with mice that were fed a calorie restricted diet, and then compared both groups to a control group. Gene chips were used to look at the expression of over 20,000 genes in liver tissue. Based on the gene chip data, it was observed that the calorie restricted group had 1,763 genes change in activity as compared to the control group, a very good indication that diet does change the expression of genes. This change in gene expression has been seen in other calorie restricted

studies [Cao 2001, Lee, 2004, Masternek 2004]. In the 3C3-OXO supplemented group, which were allowed to eat freely, 765 genes were changed in expression levels as compared to the control group. Because of the pooled data, the most interesting genes were genes that showed changes in expression in both the calorie restricted group and the 3C3-OXO supplemented group. 363 genes were shown in both groups to have "moved away" from the expression of the control group. These 363 genes are involved in lifespan extension, as is proved by the 3C3-OXO supplemented group living longer (average and maximal lifespan extension). The 363 genes were in some cases increased in activity, and in other cases decreased in activity. Comparison of the direction of the changes in gene activity between the 3C3-OXO supplemented mice and the calorie restricted mice (as compared with the control group), indicated a positive overlap of 98%. When an expression change of 1.7 was applied to the data to rule out false positives, the data showed a 100% positive overlap in gene expression change direction for both groups away from the control group.

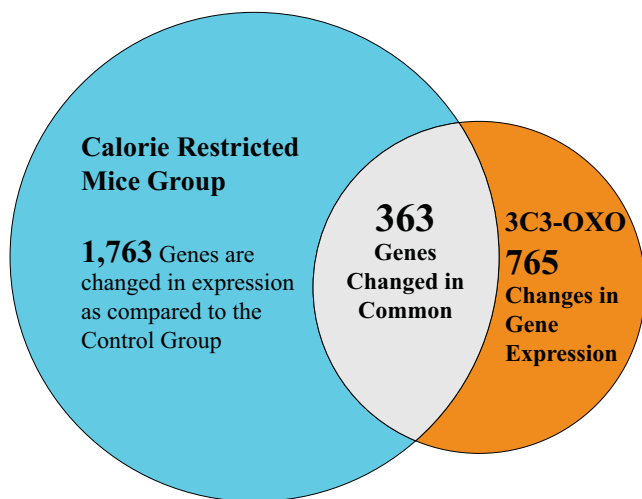
Implications for Cancer Prevention

Calorie restriction is one of the most effective means to delay the onset of cancer [Hursting, 2003]. The tie between genes expressed under calorie restriction that increase lifespan and decrease cancer proliferation has been hypothesized by Anisimov [2003]. Further to that hypothesis, it was shown that increased activation of the

Gene Symbol	Gene Title	Affy-matrix Gene Number	Change in Gene Expression Calorie Restricted to Control	Change in Gene Expression 3C3-OXO to Control	Gene function
Foxo1	forkhead box A1	2891	30% Increase	40% Increase	regulation of transcription, DNA-dependent // inferred from electronic annotation
Foxo3	forkhead box A3	13370	100% Increase	70% Increase	cell glucose homeostasis // inferred from mutant phenotype // regulation of transcription, DNA-dependent // inferred from mutant phenotype // cellular response to starvation // inferred from mutant phenotype
Foxq1	forkhead box Q1	6994	110% Increase	210% Increase	regulation of transcription, DNA-dependent // inferred from electronic annotation
Foxq1	forkhead box Q1	30006	190% Increase	220% Increase	regulation of transcription, DNA-dependent // inferred from electronic annotation

As can be seen by the table of FOXO genes that have changed, the mimic effect between mice that have 3C3-OXO supplemented diets and mice that are calorie restricted is very strong, providing a strong potential between the proven cancer reduction rates in calorie restricted animals and 3C3-OXO supplemented animals. Further work on this potential is being researched.

longevity gene FOXO3 encoded a protein to prevent cancer and predict a better outcome for breast cancer patients [Hung, 2004]. More recently, Yamamura [2006] showed that FOXO3 is needed to induce apoptosis (programmed cell death) in gastric cancer cells. Pinkston, et. al. [2006] documented that genes that increase the lifespan of *C. elegans* (worms) also inhibit tumor growth. Arden [2006] reviews the FOXO genes for potential new therapeutic targets for a broad spectrum of cancers. Her review indicated that FOXO1 is a tumor suppressor gene. She also reports that "FOXO3 can override I κ B stimulation of the cell cycle progression, proliferation and tumorigenesis in mice, further supporting FOXO3 as a candidate tumor suppressor gene."



The researchers of 3C3-OXO were excited to see the FOXO type genes were part of the 363 genes that increased in expression levels (as compared to the control group) in both the calorie restricted group and the 3C3-OXO supplemented group. (See Table)

3C3-OXO defines a new class of calorie restriction mimetic compounds, shown to have similar benefits to calorie restriction- an increase lifespan in all species tested to date, reduced glucose levels, improved glucose uptake by tissues, increased stress resistance and produces similar genomic changes. 3C3-OXO is composed of metabolites already existing in every cell of the human body- just more is added to effect a biological diode action with the mitochondria. The components of 3C3-OXO are found in excess in red apples, perhaps leading to the saying, "An apple a day...."

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**According to
the latest
nutrient
intake
survey..**

**93% of
North Americans
are not getting
enough vitamin
E from their diet.**



The fact is, the “vitamin E” supplements available in Canada are mislabeled. They contain alpha-tocopherol, and maybe a tiny bit of the other tocopherols (beta-, gamma-, and delta-tocopherols). But vitamin E is more than alpha-tocopherol. It’s more than “mixed tocopherols,” too. Vitamin E is an eight-member complex vitamin, like the B-complex. Total E includes all four tocopherols, plus the four tocotrienols in their natural ratios, as partners in the vitamin E team.

Typical “vitamin E” supplements just don’t mesh with this reality. No amount of alpha-tocopherol can fully substitute for gamma-tocopherol – and no amount of “mixed tocopherols” can make up for getting no tocotrienols in your supplement. The fact is, if you’re just getting alpha-tocopherol – or alpha with “mixed tocopherols” thrown in as an afterthought - you’re not getting “vitamin E.” You’re getting a lopsided vitamin E fraction.

Total E is Canada’s first complete, balanced E-complex vitamin first introduced in 1999. Providing all eight vitamin E molecules, just two Total E softgels a day balances the top-heavy alpha-tocopherol content in most “vitamin E” supplements or premium multivitamins.

Plus, Total E includes 30mg of coenzyme Q10, an essential synergistic partner to vitamin E’s action.