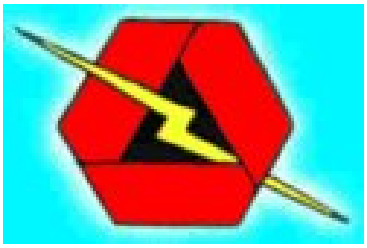

rexresearch.com

Mikhail SHCHEPINOV

Deuterated Nutrients

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http://www.telegraph.co.uk/scienceandtechnology/science/sciencenews/3529878/Heavy-water-could-help-us-live-longer.html
(27 Nov 2008)

'Heavy Water' Could Help Us Live Longer

Drinking "heavy water" enriched with a rare form of hydrogen could prolong our lives by up to ten years, it has been claimed.

By **Matthew Moore**

Mikhail Shchepinov, a former Oxford University scientist, says that the modified drink protects against dangerous chemicals known as free radicals that are known to contribute to conditions such as cancer, Alzheimer's and Parkinson's.

He also claims that foods such as steak and eggs could be enriched with the special hydrogen isotope, known as deuterium, raising the possibility of people being able to "eat themselves healthy".

His research has shown that worms live 10 per cent longer and fruitflies up to 30 per cent longer when fed on heavy water, which is slightly sweeter than normal water.

Dr Shchepinov, who runs the biotech firm Retrotope, now wants to test his technology in pet foods, and believes that it could one day be introduced to the food chain to allow humans to enjoy its benefits without taking supplements.

"We don't have to be consuming isotopes as white powder. If you take a pig and feed these things to a pig, all you need to do is consume the pig in a normal fashion," he has said.

But other scientists have warned that Dr Shchepinov's theories are far from proven. Tom Kirkwood, of Newcastle University, told the Daily Mail: "Shchepinov's idea is interesting but . . . the history in the field is cluttered with hypotheses which are only partially supported by the data."

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http://www.dailymail.co.uk/sciencetech/article-1089710/Its-time-raise-glass-heavy-water-longer-life.html
(27 November 2008)

It's time to raise a glass (of heavy water) to a longer life

by

Fiona Macrae

For centuries mankind has sought the secret of a long and healthy life.

And for centuries it seems we were looking in the wrong place. Forget exotic pills and potions, the key to prolonged life could be as simple as a glass of water. Scientists believe 'heavy water' enriched with a rare form of hydrogen could add as much as ten years to life.

And by also modifying foods, such as steak and eggs, with the hydrogen the way could be cleared to allowing us to eat and drink our way to a healthy old age.

The idea is the brainchild of Mikhail Shchepinov, a former Oxford University scientist.

It centres on fortifying the body's tissues and cells against attack and decay caused by free radicals, dangerous chemicals produced when food is turned into energy. Such 'attacks' on proteins are particularly damaging and have been linked to cancer, Alzheimer's and Parkinson's.

Dr Shchepinov's theory is based on deuterium, a naturally-occurring isotope, or form of hydrogen, that strengthens the bonds in between and around the body's cells, making them less vulnerable to attack.

He found that water enriched with deuterium, which is twice as heavy as normal hydrogen, extends the lifespan of worms by 10 per cent. And fruitflies fed the 'water of life' lived up to 30 per cent longer.

He now believes people could also benefit from the sweet-tasting water, or from deuterium-enriched 'heavy foods'.

Foods could be created by either directly supplementing them with deuterium or by enriching the feed of farm animals, this week's New Scientist reports. Dr Shchepinov said recently: 'We don't have to be consuming isotopes as white powder.

If you take a pig and feed these things to a pig, all you need to do is consume the pig in normal fashion.'

The technology was likely to be tested in pet food first, he added. Dr Shchepinov runs biotech firm Retrotope whose scientific advisers include Aubrey de Grey, a controversial ageing guru.

Dr de Grey, a 'bio-gerontologist' who leads the Methuselah Foundation, a charity which aims for 'the defeat of age-related disease and the indefinite extension of the healthy human lifespan', said the research was 'extremely promising'.

He said deuterium existed in all living matter at a certain level and it was a case of introducing it in a 'more targeted manner'. There was no radiation involved, he added.

Dr Judith Campisi, of the Buck Institute for Age Research in California, said: 'I've heard some pretty crazy ideas about how we might live longer but I'm intrigued by this.'

But Tom Kirkwood, of Newcastle University, said: 'Shchepinov's idea is interesting but . . . the history in the field is cluttered with hypotheses which are only partially supported by the data.'

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http://www.newscientist.com/article/mg20026841.800-would-eating-heavy-atoms-lengthen-our-lives.html?page=2

Would Eating Heavy Atoms Lengthen Our Lives?

by

Graham Lawton

In a back room of New Scientist's offices in London, I sit down at a table with the Russian biochemist Mikhail Shchepinov. In front of us are two teaspoons and a brown glass bottle. Shchepinov opens the bottle, pours out a teaspoon of clear liquid and drinks it down. He smiles. It's my turn.

I put a spoonful of the liquid in my mouth and swallow. It tastes slightly sweet, which is a surprise. I was expecting it to be exactly like water since that, in fact, is what it is - heavy water to be precise, chemical formula D2O. The D stands for deuterium, an isotope of hydrogen with an atomic mass of 2 instead of 1. Deuterium is what puts the heavy in heavy water. An ice cube made out of it would sink in normal water.

My sip of heavy water is the culmination of a long journey trying to get to the bottom of a remarkable claim that Shchepinov first made around 18 months ago. He believes he has discovered an elixir of youth, a way to drink (or more likely eat) your way to a longer life. You may think that makes Shchepinov sound like a snake-oil salesman. I thought so too, but the more I found out about his idea, the more it began to make sense.

The story began two years ago, while Shchepinov was working at a biotechology company in Oxford, UK, and using his spare time to read up on the latest ideas about what causes us to age.

The most widely accepted idea is the free-radical theory. This holds that our slide into decrepitude is the result of irreversible damage to the biomolecules that make up our bodies. The main agents of this destruction are oxygen free radicals, aggressive chemical compounds that are an unavoidable by-product of metabolism.

The reason oxygen radicals are so dangerous is that they have a voracious appetite for electrons, which they rip out of anything they can lay their hands on - water, proteins, fats, DNA - leaving a trail of destruction in their wake. This damage gradually builds up over a lifetime and eventually leads the body's basic biochemical processes to fail.

One of the worst types of damage is something called protein carbonylation, in which an oxygen radical attacks vulnerable carbon-hydrogen bonds in a protein (see diagram). This has been linked to many of the worst diseases of old age, including Parkinson's, Alzheimer's, cancer, chronic renal failure and diabetes (The EMBO Journal, vol 24, p 1311). Other important targets of free-radical attack are DNA and the fatty acids in cell membranes.

The human body produces legions of antioxidants, including vitamins and enzymes, that quench free radicals before they can do any harm. But over a lifetime these defence systems eventually fall victim to oxidative attack too, leading to an inevitable decline.

Many anti-ageing medications are based on supplementing the body's own defences with antioxidant compounds such as vitamin C and beta-carotene, though there is scant evidence that this does any good (New Scientist, 5 August 2006, p 40).

Shchepinov realised there was another way to defeat free radicals. While he was familiarising himself with research on ageing, his day job involved a well-established - if slightly obscure - bit of chemistry called the isotope effect. On Christmas day 2006, it dawned on him that putting the two together could lead to a new way of postponing the ravages of time.

The basic concept of the isotope effect is that the presence of heavy isotopes in a molecule can slow down its chemical reactions. This is because heavy isotopes form stronger covalent bonds than their lighter counterparts; for example, a carbon-deuterium bond is stronger than a carbon-hydrogen bond. While the effect applies to all heavy isotopes, including carbon-13, nitrogen-15 and oxygen-18 (see chart), it is most marked with deuterium as it is proportionally so much heavier than hydrogen. Deuterated bonds can be up to 80 times stronger than those containing hydrogen.

All of this is conventional chemistry: the isotope effect was discovered back in the 1930s and its mechanism explained in the 1940s. The effect has a long pedigree as a research tool in basic chemistry for probing the mechanisms of complex reactions.

Shchepinov, however, is the first researcher to link the effect with ageing. It dawned on him that if ageing is caused by free radicals trashing covalent bonds, and if those same bonds can be strengthened using the isotope effect, why not use it to make vulnerable biomolecules more resistant to attack? All you would have to do is judiciously place deuterium or carbon-13 in the bonds that are most vulnerable to attack, and chemistry should take care of the rest.

In early 2007 Shchepinov wrote up his idea and submitted it to a journal called Rejuvenation Research. Unbeknown to him, the journal's editor is controversial gerontologist Aubrey de Grey of the Methuselah Foundation in Lorton, Virginia, who is well known for supporting ideas other gerontologists consider outlandish. De Grey sent the paper out for review and eventually accepted it (*Rejuvenation Research*, vol 10, p 47).

In the paper, Shchepinov points out that there is masses of existing science backing up his ideas. Dozens of experiments have proved that proteins, fatty acids and DNA can be helped to resist oxidative damage using the isotope effect.

Shchepinov's paper brought the idea to a wider audience, including successful biotechnology entrepreneurs Charles Cantor and Robert Molinari. Impressed, they teamed up with Shchepinov to set up a company called Retrotope, with de Grey as a scientific advisor.

It was around this time that I first got in touch with Shchepinov. I'd never heard of the isotope effect, and de Grey's involvement made me cautious. But there was something in the idea that intrigued me, and I kept on coming back to it.

There were obvious objections to the idea. For one, how do you get the isotopes to exactly the sites where you want them? After all, the human body contains trillions upon trillions of chemical bonds, but relatively few are vulnerable to free-radical damage. And what about safety - swallowing mouthfuls of heavy isotopes surely can't be good for you, can it? That, of course, is how I ended up sharing a teaspoon of heavy water with Shchepinov.

Neither, it turns out, is a big problem. Some heavy isotopes are radioactive so are obviously ruled out on safety grounds - hydrogen-3 (tritium) and carbon-14, for example. Others, notably deuterium and carbon-13, are just as stable as hydrogen and carbon-12. Both occur in small amounts in nature and are a natural component of some biomolecules in our bodies (see "Heavy babies").

Deuterium and carbon-13 also appear to be essentially non-toxic. Baby mice weaned on a highly enriched carbon-13 diet are completely normal, even when 60 per cent of the carbon atoms in their body are carbon-13. Deuterium also has a clean bill of health as long as you don't go overboard. Decades of experiments in which animals were fed heavy water suggest that up to a fifth of the water in your body can be replaced with heavy water with no ill effects.

Similar experiments have been done on humans, albeit with lower levels of deuterium. One recent experiment kept humans on a low-level heavy-water diet for 10 weeks, during which their heavy-water levels were raised to around 2.5 per cent of body water, with no adverse effects (Biochimica et Biophysica Acta, vol 1760, p 730). The researchers also found that some deuterium became incorporated into proteins.

Heavy water, however, isn't completely safe. In mammals, toxic effects start to kick in around the 20 per cent mark, and at 35 per cent it is lethal. This is largely down to the isotope effect itself: any protein in your body has the potential to take up deuterium atoms from heavy water, and eventually this radically alters your entire biochemistry. You'd have to drink a vast amount to suffer any ill effects - my 5 millilitres did me no harm whatsoever - but even so, Retrotope is not advocating heavy water as an elixir of youth.

Instead, it wants to package up heavy isotopes in what Shchepinov calls "iFood". This method has huge advantages, not least because it allows the heavy isotopes to be targeted to the most vulnerable carbon-hydrogen bonds. Of the 20 amino acids used by humans, 10 cannot be made by the body and must be present in the diet. That means if you supplement your diet with essential amino acids that have already had their vulnerable bonds strengthened, your body's proteins will have these reinforced amino acids incorporated into them. Some of the building blocks of fats and DNA can also only be acquired via your diet, which means they too can be targeted using the iFood approach.

Enriched eggs

What's more, this approach ought to be completely safe, says Shchepinov. Deuterium atoms bound to carbon in amino acids are "non-exchangeable" and so don't leak into body water.

Another possibility is to produce meat, eggs or milk enriched with deuterium or carbon-13 by

feeding deuterated water or isotope-enriched amino acids to farm animals.

For now, though, iFood remains on the drawing board as nobody manufactures the right compounds. To solve that problem, Retrotope has signed up the Institute of Bio-organic Chemistry in Moscow, Russia and Minsk State University in Belarus to make customised amino acids and fatty acids. "There are a lot of good isotope chemists in Russia," says Cantor.

Another hurdle Retrotope will have to overcome is cost. At current prices, a litre of heavy water will set you back $300. "Isotopes are expensive," says Shchepinov. "But there's no need for them to be. Methods are there to extract them, but nobody wants them." Unless demand rises, there is no incentive to produce them in bulk, and this keeps the price high.

These obstacles haven't stopped Retrotope launching a research programme to test Shchepinov's big idea. A team at the Institute for the Biology of Ageing in Moscow recently fed various amounts of heavy water to fruit flies to see if it had any effect on longevity. Though large amounts were deadly, smaller quantities increased lifespans by up to 30 per cent.

It's a promising start, but it's too early to say whether the human lifespan can also be extended in this way, or how much deuterium-enriched food you would have to eat to get a beneficial effect.

"This is preliminary and needs to be reproduced under a variety of conditions," says Shchepinov. "It's possible that the flies don't like the diet, and what we're seeing is the effects of caloric restriction [the only proven strategy for extending lifespan in experimental animals]. We need to do a lot more experiments. But still..."

Retrotope has signed up some heavyweight gerontologists to join de Grey as scientific advisors, including Jan Vijg of the Albert Einstein College Of Medicine in New York and Cynthia Kenyon of the University of California, San Francisco. Kenyon recently started work on Retrotope's second round of experiments, giving a deuterium-enriched diet to nematode worms.

"It's a beautiful idea," says Vijg. "It gives us a serious chance of retarding ageing." He cautions, however, that Shchepinov's ideas hinge on free radicals being at the root of ageing. While this is still the leading theory in the field, many researchers argue that free-radical damage alone cannot account for all the biological changes that happen as we get old (Nature, vol 451, p 644).

All of which makes other mainstream researchers very sceptical. "Shchepinov's idea is interesting, but we're discovering that it only makes sense to think about ageing in terms of multiple underlying causes," says Tom Kirkwood of the University of Newcastle, UK. "The history in the field is cluttered with hypotheses which are only partially supported by the data. Therefore, it is very unlikely that his suggested mechanism will prove to be more than a small part of the much bigger picture."

Others are more positive. "I've heard some pretty crazy ideas about how we might live longer, but I'm intrigued by this one," says Judith Campisi of the Buck Institute for Age Research in Novato, California and the Lawrence Berkeley National Laboratory, who has no formal links to Retrotope. "It's very original and novel."

While Retrotope is concentrating its efforts on ageing, Shchepinov says there are other applications of the isotope effect he'd like to explore. One is shielding long-term space travellers from the effects of cosmic rays and other ionising radiation, which cause damage much like ageing.

Oxidative attack on carbon-hydrogen bonds is a problem in many other areas, from drug discovery to cancer, cosmetics chemistry and electronics. If the ageing research doesn't work out, Retrotope will try something else. "We need to sort out what works and what doesn't, and what works well enough to be commercially exploited," says Cantor. "But this is going to work somewhere, because the basic science is sound."

Sound basic science, of course, doesn't mean that Shchepinov really has cracked a problem that's been troubling humanity for millennia. Realistically, it's much more likely his insight will lead to a more prosaic application, such as stopping coloured plastics from fading in sunlight. But until he's proved wrong, I'll keep on hoping that I shared my sip of heavy water with a scientist who will be remembered long after I'm forgotten.

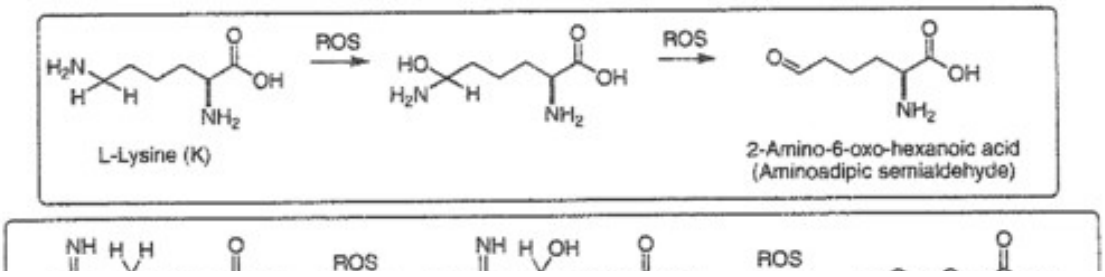
Heavy babies

The idea of using chemical isotopes to combat ageing may be new, but nature may already be onto that strategy as a way of protecting us against free-radical attack, thought to be a key cause of ageing. Babies and mice are born with much more of the isotope carbon-13 in their bodies than their mothers, and women appear to become unusually depleted in carbon-13 around the time they give birth. Both findings suggest that there is active transfer of carbon-13 from mother to fetus.One possible reason for this, suggests Mikhail Shchepinov, chief scientific officer of the biotechnology company Retrotope, which is investigating the use of isotopes to slow ageing, is that the growing fetus selectively builds carbon-13 into its proteins, DNA and other biomolecules to take advantage of the way that heavy isotopes make these molecules more resistant to free-radical attack.It would make good evolutionary sense, as many of the proteins and DNA molecules formed early on have to last a lifetime. "Every single atom in the DNA of the brain of a 100-year-old man is the same atom as when he was 15 years old," says Shchepinov (*BioEssays*, vol 29, p 1247).

WO2007102030
ISOTOPICALLY MODIFIED COMPOUNDS AND THEIR USE AS FOOD SUPPLEMENTS

2008-11-19
Classification: - international: A23L1/29; A23L1/30; A23L1/305; A23L1/29; A23L1/30; A23L1/305

Abstract --- A nutrient composition comprises an essential nutrient in which at least one exchangeable H atom is 2H and/or at least one C atom is 13C. The nutrient is thus protected from, inter alia, reactive oxygen species.



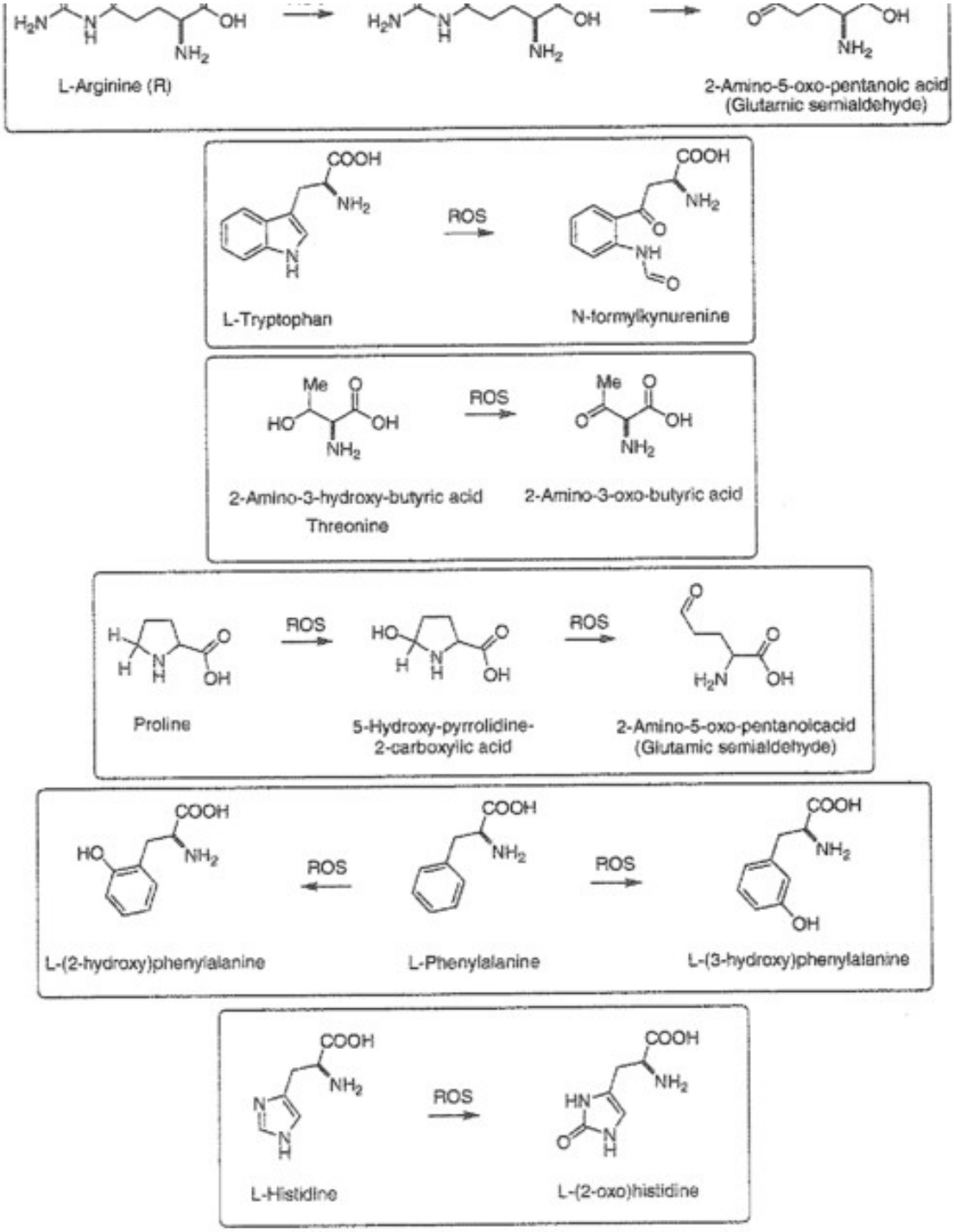
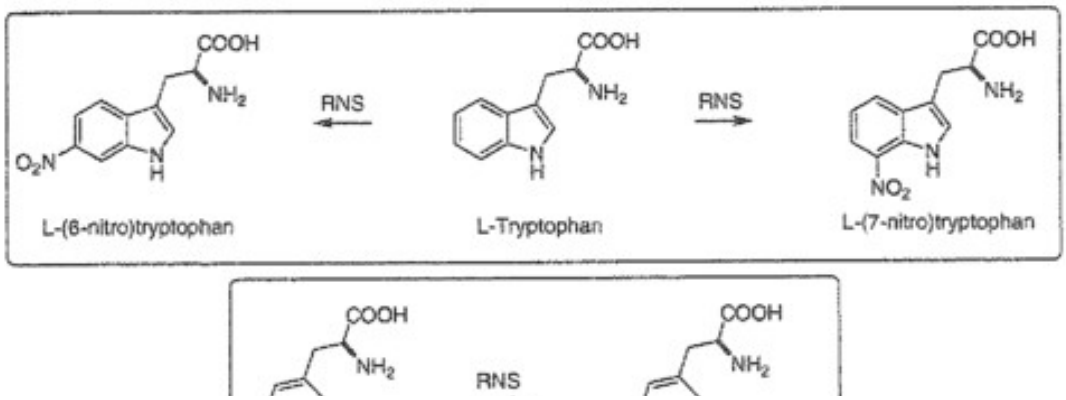

L-Arginine (R)
2-Amino-5-oxo-pentanoic acid
(Glutamic semialdehyde)
ROS
L-Tryptophan
N-formylkynurenine
2-Amino-3-hydroxy-butyric acid
Threonine
2-Amino-3-oxo-butyric acid
Proline
5-Hydroxy-pyrrolidine-
2-carboxylic acid
2-Amino-5-oxo-pentanoicacid
(Glutamic semialdehyde)
L-(2-hydroxy)phenylalanine
L-Phenylalanine
L-(3-hydroxy)phenylalanine
L-Histidine
L-(2-oxo)histidine

Figure 1


RNS
L-(6-nitro)tryptophan
L-Tryptophan
L-(7-nitro)tryptophan

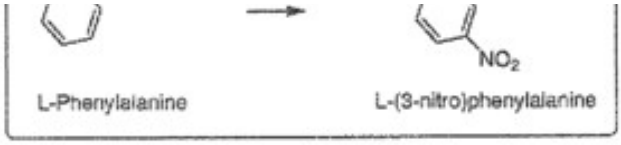


Figure 2

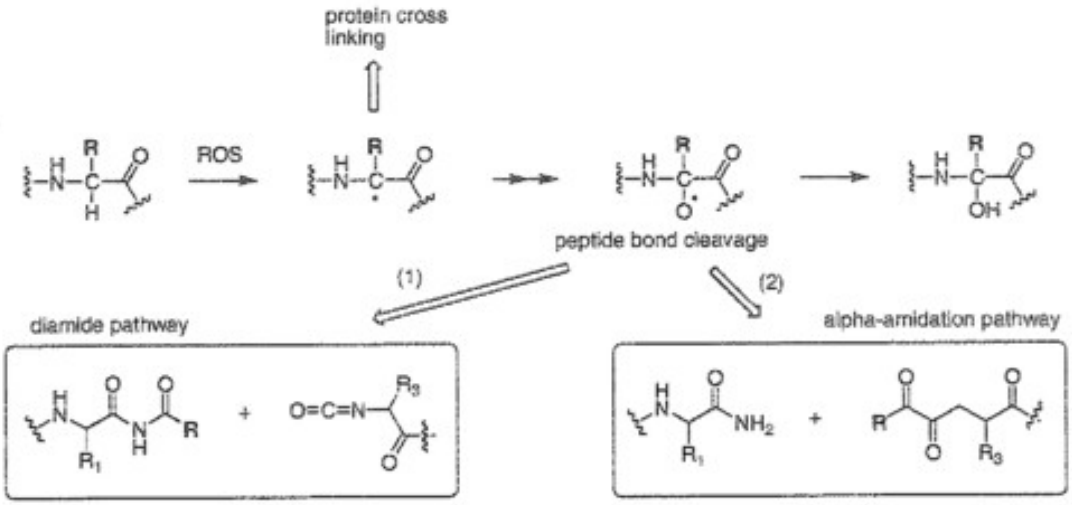


Figure 3

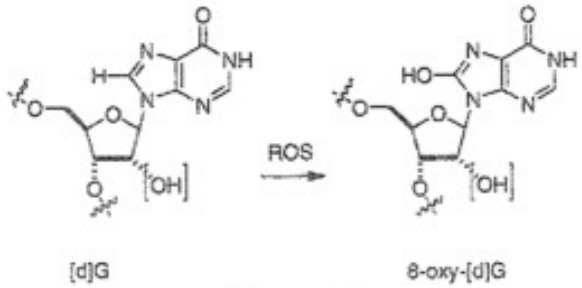


Figure 4

US2011082208

THERAPIES FOR CANCER USING ISOTOPICALLY SUBSTITUTED LYSINE

BACKGROUND

[0002] Lysyl oxidases (LOX, LOXL, LOXL2, etc.; amine oxidase family) are Cu-dependent enzymes that oxidize lysine into allysine (a-aminoadipic-d-semialdehyde) [Kagan H M. et al., J. Cell. Biochem. 2003; 88:660]. LOX have been implicated in crosslink formation in stromal collagens and elastins. LOX are elevated in hypoxic tumors and affect cell motility, tumor development and progression of metastasis [Kirschmann D A. et al., Cancer Res. 2002; 62:4478]. This elevation is mechanistically important for breast cancer metastasis and invasion as well as in other cancers including colon and esophagus [Fong S F, et al. genes

Chromosomes Cancer 2007; 6:644], and is based on the formation of Schiff-base linkages (aldehyde+amine) or aldol condensation products (aldehyde+aldehyde), allowing cancer cells to latch on to other cells/tissues. There are other mechanisms of LOX involvement into metastasis progression—for example, the recruitment of bone marrow—derived cells [Erler J T et al. Nature 2006; 440:1222-1226; Erler J T. et al., Cancer Res. 2006; 66:10238; Erler I T et al. Cancer Cell 2009; 15:35-44] for a so-called premetastatic niche formation...

[0005] A reaction important in metastatic development. It is therefore desirable to reduce the activity of lysyl oxidase in cancer. As with any cancer treatment, it is also desirable that this does not completely block the enzyme activity, so as to minimize the adverse effects of therapy on other aspects of physiology.

[0006] It is therefore desirable to reduce the activity of extracellular LOX in cancer. Some current approaches involve LOX inhibitors (e.g. ß-aminopropionitrile [Jackson L E. et al., Biochem. Biophys. Res. Commun. 1991; 179:939]), sequestration of Cu, and the use of antibodies [Erler J T et al., Nature 2006; 440:1222]. As with any cancer treatment, it is also desirable that this does not completely block the enzyme activity, so as to minimize the adverse effects of therapy on other aspects of physiology. For example, inhibition of LOX is known to cause increased elasticity of blood vessels etc., leading to aneurisms. Besides, these methods are likely to be immunogenic, as well as bringing further complications such as toxicity.

[0007] It is known that the rate of some reactions breaking or forming chemical bonds is affected by the nature of the isotopes of the atoms, which the bond links. In general, bonds terminating in a heavy isotope will be less liable to cleavage than a bond terminating in a lighter isotope. Of particular note is that bonds between hydrogen atoms and other atoms are less liable to breakage if the hydrogen is <2>H rather than <1>H. A similar effect is seen when comparing the rate of cleavage of a bond between a carbon atom and another atom, where bonds with <13>C are less liable to cleavage than bonds with <12>C. This is known as the Kinetic Isotope Effect, and is well described. Many isotopes are known to show this effect, as is described in Isotope effects in chemical reactions. (C. J. Collins, N. S. Bowman (eds.) 1970). It is known that these effects are also manifest in enzyme-catalyzed reactions, as described in. Isotope effects on enzyme-catalyzed reactions (Cleland, W. W., M. H. O'Leary, and D. B. Northrop (eds.) 1976).

SUMMARY

[0008] The KIE may be used to reduce the activity of lysyl oxidase without blocking its activity. Embodiments of this invention provide for 2,6-diamino-6,6-dideuterohexanoic acid; 2,6-diamino-5,5,6,6-tetradeuterohexanoic acid or their esters or amides, and for the use of such compounds in a treatment for a disease in which lysyl oxidase is important.

[0009] Embodiments of the invention also provide for administering supplementation by any compounds containing higher than naturally occurring prevalences of isotopes that yield stabilization of lysine via the kinetic isotope effect via incorporation of the higher than naturally occurring heavy isotope into the lysine-containing moieties in the body according to the Formulae I, II, and III described below for stabilized lysine...

US2014142059
THERAPEUTIC SUBSTANCES THAT MODULATE GENOME METHYLATION

Compounds containing nucleic acid bases or their precursors modified by enrichment at specific sites with heavy stable isotopes of elements naturally present at those sites in minute amount are useful for the treatment of diseases characterized by altered gene expression and altered pattern of epigenomic control. These compounds, when used as nutrients or in other medicinal application methods, can alter the DNA methylation pattern in a simple way through the well-understood mechanism of kinetic isotope effect (KIE). This effect could also be useful for modifying methylation kinetics in stem cell technology, cloning and as disease therapeutics.

ISOTOPICALLY MODIFIED COMPOUNDS AND THEIR USE AS FOOD SUPPLEMENTS
US8906405

A nutrient composition comprises an essential nutrient in which at least one exchangeable H atom is <2>H and/or at least one C atom is <13>C. The nutrient is thus protected from,inter alia, reactive oxygen species.

FIELD OF THE INVENTION

The present invention related to isotopically modified compounds and their use as food supplements.

BACKGROUND OF THE INVENTION

A currently accepted theory of ageing blames the irreversible changes in cell machinery and reduced efficiency of metabolic processes on the detrimental effects of free radicals and other reactive oxygen species (ROS) or reactive nitrogen species (RNS) which are normally present in the cell as part of the respiratory process. ROS and RNS oxidize/nitrate DNA, proteins, lipids and other cell components. Of these, protein oxidation, which converts arginine, lysine, threonine, thryptophan and proline into corresponding carbonyl compounds, cannot be repaired by proteases after a certain threshold number of amino acid residues have been oxidized.

The damaged protein loses its catalytic or structural activity, but proteases are unable to disintegrate heavily carbonylised strands, so that the damaged species accumulate and aggregate, clogging up cellular passages. This rust-like process gradually wears down all cellular mechanisms, slowing everything down and ultimately causing cellular death.

Apart from ageing, many diseases such as Alzheimer's, Parkinson's, dementia, cataract, arthritis, chronic renal failure, acute repiratory syndrome, cystic fibrosis, diabetes, psoriasis and sepsis, to give a few examples, are associated with increased protein carbonylation. Typically, physiological levels of protein carbonyls are at around 1 nmol/mg protein, whereas pathological levels go to 8 nmol/mg and above.

For the two molecules involved in the process of oxidative damage of proteins, i.e. an

oxidizer and its substrate, the oxidizer has been the subject of many studies aiming at neutralizing or removing it by means of increasing the number of antioxidants (vitamins, glutathione, peptides or enzymes). The substrate, e.g. amino acid (AA) residues which are converted into carbonyls, has received less attention.

One common feature of all the AA residues (except praline) vulnerable to carbonylation is that they belong to the group of essential AAs, which cannot be synthesized by vertebrata and should be ingested, e.g. consumed with food. The group includes phenylalanine, valine, tryptophan, threonine, isoleucine, methionine, histidine, arginine, lysine and leucine (arginine is essential for children of up to 5 years of age).

Oxidation of both Arg and Lys by ROS yields aminoadipic semialdehyde and proceeds through sequential replacement of ?-hydrogens with hydroxyls. Oxidation of Lys, Arg, Trp, Thr, Phe and His is shown in FIG. 1. Side-chains undergo the same transformations if these AAs are part of polypeptides/proteins. Other essential AAs undergoing ROS-driven oxidation include Leu (to 5-hydroxyleucine), Val (3-hydroxyvaline) and Ile (several products).

Other types of oxidative damages affecting essential AAs involve reactive nitrogen species (RNS). Examples are shown in FIG. 2.

Yet another process detrimental to proteins is a ROS-driven peptide bond cleavage, which is preceded by oxygen free radical-mediated protein oxidation. A hydrogen atom is abstracted from a Ca, atom of the polypeptide chain, which then leads to formation of an alkoxyl radical. This can lead either to hydroxyl protein derivative, or to peptide bond cleavage by (1) diamide or (2) a-amidation pathway. This is illustrated in FIG. 3.

Nucleic acids are not normally considered as essential components of the diet, but are also damaged by ROS. An example particularly important for the mitochondrial functioning is the formation of 8-oxy-G, as illustrated in FIG. 4. This leads to mutations in the mitochondrial genome, which is not maintained and repaired as efficiently as the nuclear genome, with detrimental consequences to the efficiency of respiratory processes in the cell. Another cause of degradation is radiation.

The kinetic isotope effect is widely used when elucidating mechanisms and rate-determining stages of chemical and biochemical reactions. The rate of reaction involving C—<1>H bond cleavage is typically 5 to 10 times faster than the corresponding C—<2>H (<2>H=D=deuterium) bond cleavage, due to the two-fold difference in the masses of H and D isotopes. The difference in reaction rates is even higher for tritium (<3>H or T) as it is 3 times heavier than hydrogen, but that isotope is unstable. The second component of the C—H bond, the carbon atom, can also be substituted for a heavier <13>C isotope, but the bond cleavage rate decrease will be much smaller, since <13>C is only a fraction heavier than <12>C. See Park et al., JACS (2006) 128: 1868-72.

Oxidation reactions are a good example of the isotope effect, as the hydrogen subtraction by an oxidizer is usually a rate-limiting step of the process. Damgaard, Biochemistry (1981) 20: 5662-69, illustrates this: the kinetic isotope effect upon V/K for (1-R)[1-<2>H2]— and (1-R) [1-<3>H2]— ethanol oxidation by liver alcohol dehydrogenase (ADH) to acetaldehyde, measured at pH 6, was 3 (D(V/K)) and 6.5 (T(V/K)), decreasing to 1.5 and 2.5 respectively at pH 9. Lower than expected rates confirm the discrete role of the non-ADH systems as alternative pathways. In vivo experiments in perfused rat liver, as reported in Lundquist et al,

Pharm. & Tox. (1989) 65: 55-62, gave the mean value of D(V/K) of 2.89. Therefore, in all cases the oxidation of deuterated ethanol was substantially slowed down.

Isotopically labelled material has been administered to animals, and also to humans, for diagnostic purposes. Gregg et al, Life Sciences (1973) 13: 755-82, discloses the administration to weanling mice of a diet in which the digestible carbon fraction contained 80 atom % <13>C. The additive was <13>C-labelled acetic acid. Tissue examination revealed no abnormalities clearly attributable to the high isotopic enrichment.

SUMMARY OF THE INVENTION

The present invention is based on the realisation that isotopic substitution can be used to synthesize a class of compounds that, when ingested, result in the formation of bodily constituents (e.g. proteins, nucleic acids, fats, carbohydrates, etc) that are functionally equivalent to normal bodily constituents but which have a greater resistance to degradative/detrimental processes, e.g. those mediated by ROS and RNS or radiation. Therefore, according to this invention, a nutrient composition comprises a nutrient composition comprising an essential nutrient in which at least one exchangeable H atom is <2>H and/or at least one C atom is <13>C.

Compounds for use in the invention are identical to normal nutrients or constituents of food except that they contain stable isotopes which, when incorporated into bodily constituents make such bodily constituents more resistant to degradative processes than they would be otherwise. They provide a method for protecting the preferred functionality of natural biomolecules; the method comprises supply of a compound in such a way that it becomes incorporated into biomolecules and in so doing confers properties on the biomolecule that protect against damaging or unwanted chemical changes.

Compounds for use in the invention may be chemically synthesized and, when ingested by an organism, are metabolized in a way that results in the incorporation of the compound into a functional biomolecule; the incorporation of the compound resulting in the biomolecule having a higher degree of resistance to damaging molecular changes than would be the case for the equivalent biomolecule that did not comprise the compound. Such compounds may act as mimics of naturally occurring precursor elements of biomolecules. They may mimic an essential amino acid. The organism is typically a plant, microbe, animal or human.

A compound for use in the invention is typically not degraded by enzymes of the P450 pathway. It can therefore accumulate in a subject for which it is essential.

DESCRIPTION OF THE INVENTION

The present invention relates to the fact that essential supplements may undergo irreversible chemical transformations such as oxidation, nitration, etc, leading to the onset of senescence or diseases. Essential food components cannot be synthesised de novo by an organism, e.g. mammal, primate or human, and therefore need to be supplied with the diet. For the purposes of this specification, a nucleic acid is essential, although it may be more properly be described as conditionally essential. Conditionally essential nutrients need to be supplied with the diet under certain circumstances.

For humans, 10 amino acids are essential, i.e. Phe, Val, Trp, Thr, Ile, Met, His, Leu, Lys and

Arg (up to the age of five). Purine and pyrimidine nucleosides are conditionally essential. Essential fatty acids are ?-3 and ?-6, while monounsaturated oleic acid is generally non-essential.

According to this invention, the proposed undesired effects such as ageing/diseases can be slowed down. The compounds consumed should be modified to slow down the undesired reactions, while still retaining their chemical identity. This can be achieved in one embodiment by substituting hydrogen atoms subjected to abstraction during oxidation/oxidative substitution at the most reactive carbon sites, or the sites known to undergo the ROS/RNS inflicted damage as illustrated on FIGS. 1-4, with deuteriums, which due to the isotope effect slow down the rate of reactions. Substituting carbons instead of or in addition to H atom substitution may require a greater degree of substitution since one does not add so much to the reaction rate decrease (D is twice the weight of H, and <13>C is less than 10% heavier than <12>C).

Depending in part of the method of preparation, a compound for use in the invention may comprise partial or total isotopic substitution. For example, deuterium substitution may be only at the one or two hydrogen atoms that are considered chemically exchangable, e.g. at OH or CH2 adjacent to a functional group. Total rather than partial <13>C substitution may often be achieved more effectively.

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NEURODEGENERATIVE DISORDERS AND MUSCLE DISEASES IMPLICATING PUFAS

BACKGROUND

[0002] 1. Field

[0003] Isotopically modified polyunsaturated fatty acids (“PUFAs”) and other modified PUFAs for treating certain diseases, particularly Alzheimer's Disease, Mild Cognitive Impairment, Frontotemperal Dementia, Amyotrophic Lateral Sclerosis and Multiple Sclerosis.

[0004] 2. Description of the Related Art

[0005] Oxidative damage is implicated in a wide variety of diseases such as mitochondrial diseases, neurodegenerative diseases, neurodegenerative muscle diseases, retinal diseases, energy processing disorders, kidney diseases, hepatic diseases, lipidemias, cardiac diseases, inflammation, and genetic disorders. Specifically, such diseases include but are not limited to Alzheimer's Disease (AD), Mild Cognitive Impairment (MCI), and Frontotemperal Dementia (FD).

[0006] While the number of diseases associated with oxidative stress are numerous and diverse, it is well established that oxidative stress is caused by disturbances to the normal redox state within cells. An imbalance between routine production and detoxification of reactive oxygen species (“ROS”) such as peroxides and free radicals can result in oxidative damage to cellular structures and machinery. Under normal conditions, a potentially

important source of ROSs in aerobic organisms is the leakage of activated oxygen from mitochondria during normal oxidative respiration. Additionally, it is known that macrophages and enzymatic reactions also contribute to the generation of ROSs within cells. Because cells and their internal organelles are lipid membrane-bound, ROSs can readily contact membrane constituents and cause lipid oxidation. Ultimately, such oxidative damage can be relayed to other biomolecules within the cell, such as DNA and proteins, through direct and indirect contact with activated oxygen, oxidized membrane constituents, or other oxidized cellular components. Thus, one can readily envision how oxidative damage may propagate throughout a cell give the mobility of internal constituents and the interconnectedness of cellular pathways.

[0007] Lipid-forming fatty acids are well-known as one of the major components of living cells. As such, they participate in numerous metabolic pathways, and play an important role in a variety of pathologies. Polyunsaturated Fatty Acids ("PUFAs") are an important sub-class of fatty acids. An essential nutrient is a food component that directly, or via conversion, serves an essential biological function and which is not produced endogenously or in large enough amounts to cover the requirements. For homeothermic animals, the two rigorously essential PUFAs are linoleic (cis,cis-9,12-Octadecadienoic acid; (9Z,12Z)-9,12-Octadecadienoic acid; "LA"; 18:2; n-6) and alpha-linolenic (cis,cis,cis-9,12,15-Octadecatrienoic acid; (9Z,12Z,15Z)-9,12,15-Octadecatrienoic acid; "ALA"; 18:3; n-3) acids, formerly known as vitamin F (Cunnane S C. Progress in Lipid Research 2003; 42:544-568). LA, by further enzymatic desaturation and elongation, is converted into higher n-6 PUFAs such as arachidonic (AA; 20:4; n-6) acid; whereas ALA gives rise to a higher n-3 series, including, but not limited to, eicosapentaenoic acid (EPA; 20:5; n-3) and docosahexaenoic (DHA; 22:6; n-3) acid (Goyens P L. et al. Am. J. Clin. Nutr. 2006; 84:44-53). Because of the essential nature of certain PUFAs or PUFA precursors, there are many known instances of their deficiency and these are often linked to medical conditions. Furthermore, many PUFA supplements are available over-the-counter, with proven efficiency against certain ailments (See, for example, U.S. Pat. No. 7,271,315 and U.S. Pat. No. 7,381,558).

[0008] PUFAs endow mitochondrial membranes with appropriate fluidity necessary for optimal oxidative phosphorylation performance. PUFAs also play an important role in initiation and propagation of the oxidative stress. PUFAs react with ROS through a chain reaction that amplifies an original event (Sun M, Salomon R G, J. Am. Chem. Soc. 2004; 126:5699-5708). However, non-enzymatic formation of high levels of lipid hydroperoxides is known to result in several detrimental changes. Indeed, Coenzyme Q10 has been linked to increased PUFA toxicity via PUFA peroxidation and the toxicity of the resulting products (Do T Q et al, PNAS USA 1996; 93:7534-7539). Such oxidized products negatively affect the fluidity and permeability of their membranes; they lead to oxidation of membrane proteins; and they can be converted into a large number of highly reactive carbonyl compounds. The latter include reactive species such as acrolein, malonic dialdehyde, glyoxal, methylglyoxal, etc. (Negre-Salvayre A, et al. Brit. J. Pharmacol. 2008; 153:6-20). But the most prominent products of PUFA oxidation are alpha, beta-unsaturated aldehydes such as 4-hydroxynon-2-enal (4-HNE; formed from n-6 PUFAs like LA or AA), 4-hydroxyhex-2-enal (4-HHE; formed from n-3 PUFAs like ALA or DHA), and corresponding ketoaldehydes (Esterfbauer H, et al. Free Rad. Biol. Med. 1991; 11:81-128; Long E K, Picklo M J. Free Rad. Biol. Med. 2010; 49:1-8). These reactive carbonyls cross-link (bio)molecules through Michael addition or Schiff base formation pathways, and have been implicated in a large number of pathological processes (such as those introduced above), age-related and oxidative stress-related conditions, and aging. Importantly, in some cases, PUFAs appear to oxidize at specific

sites because methylene groups of 1,4-diene systems (the bis-allylic position) are substantially less stable to ROS, and to enzymes such as cyclogenases and lipoxygenases, than allylic methylenes.

[0009] We have now discovered that oxidation resistant PUFAs, PUFA mimetics, PUFA pro-drugs and/or fats containing oxidation resistant PUFAs and PUFA mimetics that are useful for treating and/or inhibiting neurodegenerative disorders...