Interview

with Timo Buetler,
Nestlé Research Center

The health risks of dietary advanced glycation end-products

Dr. Timo Buetler, born in Switzerland, completed his training in biochemistry at the University of Basel and received his PhD in biochemistry from the University of Bern in 1989. After three years of post-doctoral training in molecular toxicology at the University of Washington in Seattle, he moved in 1993 to the Department of Pharmacology, Toxicology and Therapeutics at the University of Kansa Medical Center in Kansas City as Assistant Professor. After scientific appointments at Hoffmann-La Roche, the University of Bern and the University of Lausanne, he has been working at the Nestlé Research Center as Senior Scientist in Chemical Food Safety since 2003.

MNF:
Recently, Nestlé organized a workshop on the health risk of dietary glycation end-products (AGEs). The controversial contributions to this workshop are summarized in the present issue of MNF.
Dr. Buetler, are dietary advanced AGEs bad for human health?

Buetler:
If one would address this from the view point of history of exposure, one would have to conclude that most likely they do not present a risk for human health since they have been ingested for thousands of years in cooked, baked or broiled food products, in fact since humans started to use fire to cook food. Furthermore, food scientists have extensively used the Maillard reaction for the generation of flavours, colours and to modify the physicochemical properties of food.

MNF:
If not, why do AGEs evoke so many concerns in Biomedicine and Food Safety?

Buetler:
Biomedical research over the past two to three decades has observed that many diseases are associated with elevated plasma AGE levels. This is particularly true for the development of diabetic complications. While there is no doubt that the AGE body burden increases with age and in disease, the causality of AGEs, especially of dietary AGEs, in disease is still lacking.

MNF:
Does this mean that there are differences between endogenously formed and dietary AGEs?

Buetler:
Indeed, there are some significant differences. One basic difference between dietary AGEs and those generated endogenously is their chemical form. Endogenous AGEs are generally protein-bound while dietary AGEs are absorbed as free AGEs or AGEs in short peptides. While the free AGEs are rapidly cleared by the kidney, the endogenous protein-bound AGEs require degradation before they can be excreted. Research has shown that glycation can alter protein functionality, but glycated proteins are also recognized as aberrations that are degraded by the proteolytic systems. Part of the association of increased free AGEs in diabetic complications can be ascribed to the impaired renal handling of AGEs in this disease. Furthermore, our own research has shown that some physiological activities of AGEs are quite different between free and protein-bound AGEs.

MNF:
Are there any differences in biological effects between dietary and endogenous AGEs?

Buetler:
There are only limited toxicity data available for dietary AGEs. But a major problem is that adequate toxicology studies have not been performed so far. AGEs are a very heterogeneous class of chemicals and we don't even know what the most relevant AGEs to be tested are. On top of that, we have very little information on the bioavailability of AGEs to assess the exposure to individual AGEs. The only information on the toxicity of endogenous, protein-bound AGEs comes from cellular assays that are of very limited usefulness to assess their *in vivo* toxicity.

Also, it should not be forgotten that certain dietary AGEs also possess beneficial properties such as antioxidant capacity or the ability to induce cellular defence systems.

MNF:
Is there any evidence that endogenous AGEs might be toxic?

Buetler:
The association between body AGE burden and disease cannot be taken as proof of their causal implication in the disease process. Even the argument that anti-AGE drugs

slow disease progression in diabetic patients or animal models cannot be considered as full proof for causality since these drugs have multiple pharmacological properties including strong antioxidant and metal-chelating activities. Interestingly, disease-protective effects were observed when pure antioxidants that were not designed as anti-AGE drugs were used. These antioxidants also reduced the AGE body burden. If one considers that AGE formation is favoured in an aqueous environment containing oxygen, such as in the human body, one can reasonably propose that AGEs may rather be markers of oxidative stress than disease causing agents. The beneficial effects of antioxidants in disease prevention are unchallenged and their administration may result in a parallel decrease in AGE formation.

MNF:
In literature, several mechanisms describe how AGEs could exert their biological activity. What is your opinion on these mechanisms?

Buetler:
The most discussed mechanism involves the interaction of AGEs with the receptor for AGEs, RAGE, that is linked to inflammation and oxidative stress. In my view, oxidative stress is probably the most important factor affecting AGE formation in non-food systems, but probably also in food systems. AGE formation and diseases can both be prevented by antioxidants and there is good reason to believe that AGEs are formed in parallel during oxidative stress and may perhaps be regarded as markers of oxidative stress. However, regarding the mechanisms of the AGE-RAGE interactions, there are many unsolved issues.

MNF:
What are these unsolved questions?

Buetler:
For example, the evidence in the literature is not consistent regarding inflammatory cell stimulation by AGEs *via* RAGE. Some publications showed that AGEs may not be able to activate RAGE, even though they bind tightly to RAGE. In addition, RAGE activation is generally not an isolated event *in vivo* since it is normally linked to other inflammatory signals such as TNF-α, interleukins, *etc*. These act *via* their own receptors, but converge on the same down-stream events as RAGE, *i.e*. activation of inflammatory genes. In general, the activation of inflammatory signalling induced by TNF-α, IL-6, Il-1β or LPS *via* their respective receptors are predominant compared to that induced by RAGE activation. Furthermore, AGEs interact not only with RAGE but with a multitude of other receptors.

MNF:
You mention other receptors, what is known about these receptors?

Buetler:
These receptors include AGE-receptors, CD36, scavenger receptors and probably others that are as yet ill-defined. The scavenger receptors are in general thought to bind AGEs, resulting in their degradation. Then, it has to be taken into account that AGEs are competing with endogenous RAGE ligands for RAGE binding and these interactions have not really been considered yet. In summary, the AGE-RAGE interactions are very complex and far from being understood. AGEs are multi-receptor ligands, RAGE is a multi-ligand receptor and these interactions come into play in a cellular context involving other inflammatory signaling molecules activating their own receptors. It is indeed a gigantic task to unravel this puzzle.

MNF:
Besides receptor-mediated processes, are there other mechanisms that could explain the biological effects of AGEs?

Buetler:
It is important to stress that the toxicity of AGEs is unproven. AGEs probably have few other effects as they are rather unreactive. However, if one considers that AGEs are formed from reactive precursors under the influence of oxidative stress, perhaps the focus should be shifted to these. The dicarbonyl intermediates are very reactive and are formed from breakdown products of the Maillard reaction but also from side products of glycolysis and from lipid peroxidation. These compounds have the potential to modify cellular proteins that could lead to alterations in their function that could ultimately lead to a disruption of cellular homeostasis.

MNF:
What would be the consequences for science in this field?

Buetler:
Clearly more research is needed. We need to identify which food AGEs are most relevant for the biological effects. We need to understand their bioavailability and metabolism. And most importantly, the issue of AGE toxicity should be addressed by performing appropriate toxicological studies. To assess the toxicity of non-food AGEs, we also need more information on the potential toxic mechanisms. Together, this should ultimately enable us to perform a safety assessment and fix intake levels, if necessary. We should also do more research on the effect of the reactive dicarbonyl intermediates to understand their role in toxicity. Because of the reported beneficial properties of certain AGEs, we should also conduct risk-benefit analyses to evaluate the safety of individual AGEs.

And finally, it should be noted that tests should also include AGE-related molecules, such as advanced lipoxidation end-products or ALEs. These are similar to AGEs and some

AGEs are formed both during lipid peroxidation and from the Maillard reaction. CML is a good example of this. What has been said for the AGEs also applies for the ALEs, *i. e.* they are end-products and as such relatively unreactive. And as for AGEs, their importance probably lies in the formation and reactivity of the precursors. And again as for AGEs, lipid peroxidation only occurs in the presence of oxidative stress. Thus, by reducing oxidative stress, both lipid peroxidation and formation of AGEs/ALEs can be attenuated resulting in host protection where diet plays an important role.

MNF:
And for consumers?

Buetler:
Although AGEs/ALEs are present in the diet, I strongly believe that dietary AGEs are of little health concern. Moreover, the presence of dietary antioxidants most likely largely outweighs the potential minor influence these compounds may have on human health. Thus, although we are constantly exposed to AGEs, ALEs and their precursors, we should not worry too much about them. As long as we eat a balanced diet, we ingest large amounts of antioxidant power to fight against oxidative stress, the reactive intermediates and their toxicity. After all, we have lived well with AGEs/ALEs for many thousands of years.

MNF:
Dr. Buetler, thank you very much for this interview.

Interview by Monika Pischetsrieder

