Protein modification by advanced Maillard adducts can be modulated by dietary polyunsaturated fatty acids

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Abstract
Advanced Maillard adducts, such as ε-(carboxymethyl)lysine and ε-(carboxyethyl)lysine, can be formed efficiently in vitro from both peroxidation of polyunsaturated fatty acids and glycolysis intermediates. In an attempt to differentiate the in vivo influence of the two pathways in these modifications, Wistar rats were chronically fed with specially designed diets rich in saturated or unsaturated fats. The degree of fatty acid unsaturation of all analysed organs (liver, kidney, brain) was altered by these dietary stresses. Protein glycoxidative and lipoxidative modifications were measured by GC/MS. In accordance with fatty acid profiles, concentrations of ε-(malondialdehyde)lysine in these tissues were significantly increased in animals fed the unsaturated fat diet. In contrast, ε-(carboxymethyl)lysine and ε-(carboxyethyl)lysine concentrations were strongly dependent on the tissue analysed; although the unsaturated fat diet increased their levels significantly in brain, levels were unchanged in kidney and decreased in liver. These later results could be interpreted on the basis that polyunsaturated fatty acids decrease the expression of several glycolytic enzymes in liver. Globally, these data suggest that tissue-specific metabolic characteristics play a key role in the degree of cellular protein modification by Maillard reactions, e.g. by modulation of the concentration of glycolysis intermediates or via specific defensive systems in these organs.

Non-enzymatic modifications of proteins
Non-enzymatic modifications of proteins can arise from direct exposure to reactive oxygen, chlorine or nitrogen species, generating oxidative products such as glutamic semialdehyde and aminoadipic semialdehyde [1], or from reaction with low-molecular-mass reactive carbonyl compounds derived from amino acids, carbohydrates or PUFAs (polyunsaturated fatty acids), such as glyoxal, glycolaldehyde, MGO (methylglyoxal), MDA (malondialdehyde) and hydroxynonenal, among others [2]. These carbonyl compounds could react primarily with lysine, arginine and cysteine residues, leading to the formation of both adducts [AGEs/ALEs (advanced glycation/lipoxidation end products)] and cross-links in protein [2]. Examples include the formation of CML [ε-(carboxymethyl)lysine] [3], CEL [ε-(carboxyethyl)lysine] [4], MDA-lys [ε-(malondialdehyde)lysine] [5], argpyrimidine and lysine-lysine cross-links. Less reactive carbonyl compounds, such as glucose and other reducing sugars, can also react with proteins, forming intermediate Amadori adducts that may evolve to form stable AGES, such as pentosidine and CML [2].

Reactive carbonyl species are formed in a variety of metabolic reactions [2]. Some are generated by non-oxidative pathways, such as the formation of MGO by the spontaneous decomposition of triose phosphates or during anaerobic metabolism of acetone and amino acids. Other carbonyl species derive from oxidative reactions. For example, glyoxal, MGO and glycolaldehyde are formed during the autoxidation of carbohydrates. Lipid peroxidation reactions can also produce glyoxal and MGO. The carbonyl compounds dehydroascorbate, acrolein and MGO are also produced during the oxidation of ascorbate, hydroxyamino acids and PUFAs respectively.

In vivo, the relative significance of these different pathways of non-enzymatic protein modification will depend on the interplay between favouring factors, such as the oxidative status and the concentration and reactivity of carbonyl species or their precursors. Also important are the mechanisms of protection against damage mediated by the Maillard reaction through (i) enzymatic inactivation, (ii) the concentration of endogenous amines that can trap carbonyl compounds, (iii) the cellular removal and degradation of modified proteins and (iv) renal clearance [2,6–8].

PUFAs
PUFAs are essential components that confer fluidity, flexibility and selective permeability to cellular membranes. PUFAs regulate several cellular and physiological processes, mainly by influencing cellular membrane lipid composition,
metabolism and signal–transduction pathways, and by direct control of gene expression. PUFAs, specifically the \( n-3 \) and \( n-6 \) series, may regulate the steady-state level of non-enzymatic modification of proteins via at least two general mechanisms. One involves the control by PUFAs of metabolic pathways by governing the DNA-binding activity and nuclear abundance of selected transcription factors responsible for regulating the expression of genes encoding key regulatory proteins of lipid and glucose metabolism [9]. Thus PUFAs are homoeostatically regulated in a tissue-specific way [10,11]. The other mechanism involves the sensitivity of PUFAs to oxidation. The presence of a methylene group between two double bonds renders a tissue-specific way [10,11]. The other mechanism involves the sensitivity of PUFAs to oxidation. The presence of a methylene group between two double bonds renders a FA (fatty acid) sensitive to free radical-induced damage, with sensitivity to oxidation increasing exponentially as a function of the number of double bonds per FA molecule [12]. Consequently, the high concentration of PUFAs in phospholipids not only makes them major targets for reaction with oxidizing agents, but also enables them to participate in long free radical chain reactions.

**PUFAs and non-enzymatic modifications of proteins**

However, there is a lack of **in vivo** studies dealing with the possible effects of the modification of tissue FA unsaturation on specific markers of non-enzymatic protein modification. In an attempt to overcome this limitation, the degree of membrane unsaturation was successfully modified in the brain, liver and kidneys of rats fed with specially designed semi-purified diets differing in their content of saturated and unsaturated FAs. In these three tissues, the double bond index (DBI; calculated from the FA composition), the specific constituents of protein carbonyls (glutamic semialdehyde and aminoadipic semialdehyde), the lipoxidation-derived protein marker MDA-lys and the mixed AGE/ALE compounds CML and CEL were measured (Table 1).

| Parameter | Liver | | | Kidney | | | Brain | | |
|-----------|-------|-------|-------|-------|-------|-------|-------|-------|
|           | SAT   | UNSAT | \( P \) | SAT   | UNSAT | \( P \) | SAT   | UNSAT | \( P \) |
| DBI       | 136 ± 3 | 226 ± 4 | 0.0001 | 189 ± 1 | 215 ± 1 | 0.0001 | 178 ± 2 | 192 ± 1 | 0.003 |
| GSA (\( \mu \text{mol/mol} \)) | 3309 ± 271 | 2828 ± 140 | NS | 19 678 ± 712 | 19 413 ± 852 | NS | 9852 ± 528 | 10 568 ± 1019 | NS |
| AAS (\( \mu \text{mol/mol} \)) | 52 ± 6 | 70 ± 12 | NS | 75 ± 10 | 70 ± 7 | NS | 68 ± 5 | 102 ± 12 | 0.04 |
| CML (\( \mu \text{mol/mol} \)) | 802 ± 71 | 570 ± 24 | 0.02 | 1276 ± 152 | 1025 ± 50 | NS | 762 ± 29 | 987 ± 76 | 0.03 |
| CEL (\( \mu \text{mol/mol} \)) | 461 ± 14 | 369 ± 16 | 0.003 | 237 ± 36 | 248 ± 18 | NS | 428 ± 31 | 520 ± 12 | 0.03 |
| MDA-lys (\( \mu \text{mol/mol} \)) | 213 ± 17 | 321 ± 33 | 0.03 | 138 ± 26 | 375 ± 79 | 0.03 | 197 ± 20 | 499 ± 60 | 0.001 |

Globally, higher levels of \( n-3 \) PUFAs and lower levels of \( n-6 \) PUFAs were observed in the brain, liver and kidneys of the group fed on a diet rich in unsaturated fats (UNSAT group) in relation to the group fed on a diet rich in saturated fats (SAT group). As a result, the final DBI was significantly higher in the brain (by 8%), kidney (by 14%) and liver (by 65%) of the UNSAT compared with the SAT group. Among the studied markers of protein modification, MDA-lys was the one showing most consistent changes among organs, since it was higher in the UNSAT than in the SAT group in the three organs studied. These increases reached 150–170% in brain and kidney and 50% in liver. In the brain, almost all the other protein markers were also present at higher levels in the UNSAT than in the SAT group, including aminoadipic semialdehyde (by 51%), CML (by 30%) and CEL (by 21%), while significance was not reached in the case of glutamic semialdehyde, which did however show a trend to increase (by 7%). In the liver, CML and CEL were significantly lower (20–29%) in the UNSAT compared with the SAT group, whereas aminoadipic semialdehyde and glutamic semialdehyde did not differ between these groups. Finally, in the kidney, in contrast with the marked changes observed in MDA-lys, no significant differences between groups were observed for the other markers analysed.

Thus the FA double bond content of rat brain, liver and kidney was successfully manipulated by chronic feeding with diets differing in FA unsaturation. This allowed us to investigate if the degree of unsaturation of tissue FA substrates affects not only lipidic components, but also non-enzymatic modification of proteins **in vivo**.

Among the different protein markers measured, MDA-lys was the one that was most sensitive to the degree of tissue FA unsaturation. **In vivo** levels of MDA-lys were markedly higher in the UNSAT than in the SAT group for all organs studied, whereas this was not the case for any of the other protein markers measured. This agrees with a

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Table 1 | DBI and markers of oxidative, glycoxidative and lipoxidative stress in liver, kidney and brain proteins of rats fed diets rich in saturated (SAT) or unsaturated (UNSAT) fat

Male Wistar rats of 8 weeks of age were used. The duration of dietary stress was 7 weeks. Results are means ± S.D. from six different animals per group. NS, no significant difference was found between groups. GSA, semialdehyde; AAS, aminoadipic semialdehyde. Protein modification markers are expressed in \( \mu \text{mol/mol of lysine} \). DBI was calculated as:

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\text{DBI} = \left[ \left( 1 \times \Sigma \text{mol\% monoenoic} \right) + \left( 2 \times \Sigma \text{mol\% dienoic} \right) + \left( 3 \times \Sigma \text{mol\% trienoic} \right) + \left( 4 \times \Sigma \text{mol\% tetraenoic} \right) \right] \\
+ \left( 5 \times \Sigma \text{mol\% pentaenoic} \right) + \left( 6 \times \Sigma \text{mol\% hexaenoic} \right) 
\]
previous study from our laboratory in which the increases in MDA-lys in rat heart mitochondria [13] with unsaturated fat were higher than those observed for protein carbonyls or CML. These results were expected, as MDA is a major product of the peroxidation of highly unsaturated FAs, which makes the protein MDA-lys concentration directly lipid peroxidation-dependent. Brain CML and CEL levels also increased in the UNSAT group in our present investigation. The lower sensitivity of brain CML and CEL to the DBI (when compared with MDA-lys) may be explained by the mixed origin of CML, i.e. from both lipid peroxidation and glycoxidation [3], and by the major contribution of carbohydrate oxidation for CEL. Furthermore, the increases in CML and CEL in the UNSAT group were limited to brain tissue, in contrast with MDA-lys. In the case of the liver, decreases in CML and CEL were observed in the UNSAT group. It is known that in liver, PUFAs – specifically n − 3 PUFAs – cause redistribution of glucose to enhanced glycogen synthesis and away from glycolysis by decreasing the expression of many genes coding for glycolytic enzymes [14]. This would decrease the availability of triosephosphate intermediates, which contribute (CML) or mainly determine (CEL) the formation of CML and CEL through glycoxidation reactions. Thus a PUFA-induced lowering of those hepatic carbohydrate intermediates is probably a major factor in the decreases in CML and CEL specifically observed in liver tissue. Nevertheless, putative increases in the hepatic catabolism of these adducts cannot be ruled out. In any case, tissue-specific changes in the turnover of modified proteins driven by the different FAs could be important for all of the protein oxidation markers measured in our study, since the magnitude of their changes across tissues was not a simple direct function of the change in DBI. For instance, the DBI increased to a greater extent in the UNSAT group in the liver than in the other two organs, whereas the largest increases in MDA-lys were observed in brain and kidney. Consequently, under physiological conditions, the steady-state level of a given marker results from a combination of tissue-specific factors such as oxidative stress, substrate concentration and protein turnover, among others.

Conclusions
In summary, the results of this and a previous investigation from our laboratory demonstrate that increasing tissue FA unsaturation can increase various markers of protein modification (notably MDA-lys) in tissues in vivo. This is especially evident in heart and brain, whereas in liver and kidney increases, lack of change or even decreases can be observed depending on the parameter measured. These findings are relevant to the present debate about the levels of dietary intake of n − 3 and n − 6 PUFAs that are optimum for health, which must be enough to protect against some diseases without dangerously increasing non-enzymatic modification of tissue macromolecules.

References

Received 3 July 2003