



SDI FINAL EVALUATION FORM 1.1

PART 1:

Journal Name:	Annual Research & Review in Biology
Manuscript Number:	2013_ARRB_8763
Title of the Manuscript:	Current Methods Used in the Protein Carbonyl Assay

PART 2:

FINAL EVALUATOR'S comments on revised paper (if any)	Authors' response to final evaluator's comments
The revised version includes new information and recently developed techniques to quantify protein carbonyls. This said, there are still some errors that authors should ammend. I've highlighted them in red. Corrections,when proposed, are in green 1. Tamarit J., de Hoogh A., Obis E., Alsina D., Cabiscol E., Ros J. Analysis of oxidative stress-induced protein carbonylation using fluorescent hydrazides. <i>Proteomics</i> 2012; 75:3778-3788. Should be <i>J. Proteomics</i> 2. Recently, Wehr and Lenvine [42]. Should be <i>Levine</i> 3. Why the authors repeat in the same paragraph the definition of AGEs? Please rewrite and clarify Glycation is one of the major causes of spontaneous damage to cellular and extracellular proteins in physiological systems; <i>advanced glycation end products (AGEs)</i> form when proteins interact with aldose sugars for an extended period of time (weeks, months). The initial glycation reaction is followed by a cascade of chemical reactions resulting in the formation of intermediate products (Schiff base, Amadori and Maillard products) and finally to a variety of derivatives named <i>advanced glycation end products (AGEs)</i>. In hyperglycemic environments and in natural aging, AGEs are generated in increased <i>concentrations</i>; <i>their level</i> can be evaluated in plasma due to the fact that they are fluorescent compounds. Please correct: <i>concentrations</i> and their <i>levels</i> 4. Line 238: Please correct: The quantification of <i>protein carbonyls</i> 5. This sentence is long and not strucutred, meaning that it is very difficult to follow. Please reflect and rewrite for clarity <i>Madian and Regnier [48] reported the first proteomic-based identification and characterization of oxidized and carbonylated proteins in human plasma using the isolation from the plasma with avidin affinity chromatography subsequent to biotinylation of carbonyl groups with biotin hydrazide and sodium cyanoborohydride reduction of the resulting Schiff's bases</i> 6. Line 323: Please correct : <i>With the iTRAQ method it was possible</i> to identify over 200 carbonylated proteins from rat skeletal muscle mitochondrial protein isolates [49]. 7. Please correct: Jha R, Rizvi I. Carbon~~i~~ formation in erythrocyte membrane proteins during aging in humans.	1. Endorsed. The reference was updated. (line 460) 2. Endorsed. The name was corrected (line 269) 3. Indeed, the definition was twice. So, we corrected the paragraph, accordingly: Glycation is one of the major causes of spontaneous damage to cellular and extracellular proteins in physiological systems; <i>advanced glycation end products (AGEs)</i> form when proteins interact with aldose sugars for an extended period of time (weeks, months). The initial glycation reaction is followed by a cascade of chemical reactions resulting in the formation of intermediate products (Schiff base, Amadori and Maillard products) and finally to a variety of derivatives named <i>AGEs</i>. In hyperglycemic environments and in natural aging, AGEs are generated in increased <i>concentrations</i>; <i>their levels</i> can be evaluated in plasma due to the fact that they are fluorescent compounds. Endorsed. The correction was made in the text(lines 42-43) 4. Endorsed. The line 238 was corrected. 5. Endorsed. The sentence was changed as following: Madian and Regnier [48] reported the first proteomic-based identification and characterization of oxidized and carbonylated proteins in human plasma. The method used implies following steps: isolation from the plasma with avidin affinity chromatography, then biotinylation of carbonyl groups with biotin hydrazide followed by sodium cyanoborohydride reduction of the resulting Schiff's bases. 6. Endorsed. The sentence was updated accordingly (line 326) 7. Endorsed. The reference was corrected.(line 398)