Published in final edited form as:

Nutr Metab Cardiovasc Dis. 2013 December ; 23(0 1): . doi:10.1016/j.numecd.2012.05.006.

Assessment of Old and New Proteins

Matthew L. Johnson and K. Sreekumaran Nair

Mayo Clinic Division of Endocrinology 200 First Street SW Joseph 5-194 Rochester MN, 55905

Matthew L. Johnson: johnson.matthew3@mayo.edu; K. Sreekumaran Nair: nair@mayo.edu

Abstract

Protein modifications and the accumulation of those proteins are implicated in a host of diseases from Parkinson's and Alzheimer's to both insulin independent and insulin dependent diabetes mellitus. Accumulation of irreversibly modified proteins occurs when the degradation rate of proteins is reduced or the rate of modification increases. Although the synthesis rates of individual proteins *in vivo* have been extensively studied the methodology to measure degradation rates of individual proteins *in vivo* remains to be well developed. However, the ability to measure the relative age of a particular protein pool in relation to the quality of the pool (amount of damage) is a recent advance. This brief review describes a novel methodology to simultaneously measure the synthesis rate of individual proteins along with the accumulation of oxidative damage to those proteins *in vivo*. The results of a recent investigation on individuals with type 1 diabetes mellitus are described. Accelerated damage to *de novo* synthesized ApoA1 is shown during short-term insulin cessation, which has potential clinical implications. Future implications of the novel method in diabetes and aging are also discussed.

Keywords

protein; synthesis; turnover; isotope tracer; individual proteins

Introduction

The cachectic nature of type I diabetes (T1D) during the pre-insulin era is a striking example of protein degradation exceeding protein synthesis. It is now known that insulin's therapeutic affect on protein metabolism in T1D is a combination of decreasing protein breakdown relatively more than decreasing protein synthesis (1–3), therefore restoring whole body protein balance. The sarcopenia of aging is a second example of mismatched protein synthesis and degradation. Starting in the third decade of life a gradual loss of body protein at the rate of approximately ~4% per decade (4) significantly contributes to physical frailty and cardiometabolic risks in elderly people (5). As sedentary people become older there is a progressive decrease in the rate of muscle protein synthesis. Protein degradation must decrease as well in order to explain the gradual loss of body protein observed in the sarcopenia of aging, however the ability to measure individual protein breakdown *in vivo* is limited. Stable isotopes can be used to determine protein breakdown for the whole body (6), across tissues (7), and mixed muscle proteins (8), but the ability to measure the degradation rate of protein sub-fractions or individual proteins has not been feasible to date. Research on

© 2012 Elsevier B.V. All rights reserved.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

the pathways regulating protein degradation, the autophagy-lysosome and ubiquitin-proteasome pathways, suggests that age decreases the activity of these pathways (9–11). Importantly, maintenance of autophagy pathways maintains the function of important organelles such as the mitochondria (12) highlighting the biological importance of protein turnover.

Both aerobic and resistance exercise programs are known to effectively counteract many aspects of the sarcopenia of aging (4,13). Although both aerobic (4) and resistance exercise (14) increase muscle protein synthesis only resistance exercise increases muscle mass, this suggests that aerobic exercise also increases muscle protein degradation (15). It is important to appreciate that in both T1D and the sarcopenia of aging the mechanism of treatment affects both protein synthesis and degradation therefore having an effect on the turnover rate of those proteins.

Maintaining the Quality of the Proteome

Each protein does not exist as a homogenous pool within a tissue, rather as a heterogeneous mix of isoforms of the same protein separated by a single variable, the age since ribosomal translation (Figure 1). A protein pool therefore is a mixture in continuum of proteins at various ages after its synthesis. The age of a protein relative to its function is increasingly becoming important to understand. In diabetes for example, growing evidence indicates that oxidative stress and the effects of oxidative damage are major contributors to the pathogenesis of diabetic complications (16,17). Proteins are susceptible to oxidative damage through irreversible modifications such as oxidation, carbonylation or deamination (18,19). Thus a proteome exposed to high levels of oxidative stress, such as T1D, may experience a relative increase in isoforms of oxidatively damaged proteins compared to a low oxidative stress environment. The oxidative environment is also determined by the endogenous antioxidant defense system; with a more robust antioxidant defense system reducing the chance of oxidative damage (20–22). In aging, both a decrease in the endogenous antioxidant defense system coupled with a decrease in protein turnover increases the rate and average length of time a protein can be modified before degraded from the proteome (4,14,23,24), thus increasing the likelihood that the accumulation of damaged or modified proteins could occur.

Growing evidence suggests that the quality of protein does matter in the pathophysiology of certain diseases. In the central nervous system for example, disturbances in the metabolism of proteins are implicated to contribute and in some cases cause neurodegenerative diseases such as Parkinson disease (25), Alzheimer disease (26), and Creutzfeldt-Jakob disease (27). In fact, recent advances in Alzheimer's research now allows investigators to investigate the synthesis and clearance rates of cerebrospinal fluid *in vivo* to isolate changes in the accumulation of amyloid- β in the brain (28). Likewise, oxidative damage of plasma proteins is associated with many chronic complications of T1D (19,29–32). In aging, exercise training results in improvements in muscle performance that often exceed increases in muscle area (33), indicating an increase in quality and intrinsic function of muscle in response to increased turnover (4). In each of those situations the ability to simultaneously measure the synthesis rates of individual proteins along with the accumulation of environmental damage to those proteins may provide valuable insights into the pathophysiology of T1D and aging.

Measuring Old and New Proteins – Developing the Method

Developing a method to measure the relative age of individual proteins started with the understanding that in T1D meticulous glycemic control improves microvascular

complications, but does little to improve macrovascular complications (16). While trying to understand this inconsistency it was realized that T1D individuals often have normal or even high levels of high-density lipoprotein cholesterol (HDL), but the most frequent cause of death is cardiovascular disease (34). Yet non-diabetic individuals show a clear inverse relationship between HDL levels and cardiovascular disease (35). Does T1D somehow increase the susceptibility of liver derived proteins to oxidative damage that could provide a mechanism for altered function and subsequently explain the dissociation between HDL levels and cardiovascular disease?

Insulin deprivation in T1D increases whole body protein degradation, as indicated by both increased leucine flux and increased leucine oxidation (1). The findings of increased amino acid degradation and oxidation during insulin deficiency are consistent with the observed anti-catabolic effect of insulin treatment on protein metabolism. Surprising, however, is that insulin deprivation also increases protein synthesis, as indicated by increased non-oxidative leucine flux. While the whole body net effect of insulin deprivation is a catabolic state, the increased non-oxidative disposal of amino acids (phenylalanine and leucine) is accounted for by a concomitant increase in synthesis across the splanchnic region (2).

During insulin deprivation in T1D high splanchnic protein synthesis is accompanied by increased whole body oxygen consumption. We (36) have shown that the increased oxygen consumption during insulin deprivation is related to elevated glucagon levels. Moreover, inhibition of glucagon secretion by somatostatin during insulin deprivation in T1D reverses the increased oxygen consumption while subsequent re-introduction of glucagon restores the higher oxygen consumption of insulin deprivation. Because the liver is the main site of glucagon action, it is likely that much of the increased oxygen consumption during insulin deprivation occurs in the liver. Glucagon is known to enhance energy demanding processes including gluconeogenesis, urea production, and synthesis of liver derived proteins such as fibrinogen (37). The combined effects of increased splanchnic amino acid metabolism along with elevated glucagon, glucose, and oxygen consumption in the liver are likely to increase the mitochondrial membrane potential that, in the presence of impaired ATP synthesis would result in mitochondrial superoxide production. Others have found that hyperglycemia increases superoxide production in endothelial cells by increases in the mitochondrial membrane potential during impaired ATP production (38). In isolated mitochondria from T1D human muscle, maximal ATP synthesis is reduced under insulin deprivation (39) that, if is also true in the liver as an increased presence of lipid oxidation products suggests (40) such impairment could increase oxidative damage to newly synthesized liver proteins as a result of each glycemic excursion.

In addition to the potential for increased liver oxidative stress, plasma levels of oxidative stress are well documented in diabetes and hyperglycemia. For example biomarkers of oxidative stress such as allantoin and isoprostane levels are elevated in T1D individuals (30,41). Potential sources of circulating superoxide production include xanthine oxidase (42) and NAD(P)H oxidases (43). The combination of increased amino acid and glucose metabolism in conjunction with plasma and possibly liver specific oxidative stress creates an environment likely to rapidly damage liver derived circulating proteins. Increased oxidative damage should be measureable after such an event in liver specific proteins such as apolipoprotein A-1 (ApoA-1), the major protein component of HDL. Further this oxidative damage should occur more rapidly during the insulin-deprived state in comparison to insulin treated state and because the damage will accumulate over time older isoforms will exhibit increased oxidative damage in comparison to newer isoforms in both states. Until recently no methodology existed to measure the relative age of proteins, and thus, hypotheses related to the accumulation of aged proteins could not yet be tested.

Two-dimensional gel electrophoresis (2DGE) of tissue or body fluids allows for the separation of numerous protein gel spots of the same protein identity (44), and often these spots exist as charge variants and thus present as a train of spots horizontally adjacent to one another on the gel. Each adjacent spot has a similar molecular weight but different isoelectric point (pI) due to specific, and in some cases irreversible modification. This phenomenon is known to occur to proteins specific to various diseases such as ApoA-1 and fibrinogen, both synthesized in the liver (45). This method was further developed in our laboratory to simultaneously measure the synthesis rates of individual skeletal muscle mitochondrial proteins (46).

We hypothesized that using 2DGE technology along with amino acid stable isotope and mass spectrometry that the different protein isoforms in the horizontally adjacent spot trains (see figure 2) were related to the age of the protein (44). To test this hypothesis we used the principles of tracer incorporation into proteins where it is reasonable to assume that during an intravenous infusion of an amino acid tracer such as L[ring- $^{13}\text{C}_6$]phenylalanine, both tracer and tracee will be incorporated into proteins synthesized during the course of the infusion or after the infusion. However, no tracer would be incorporated into proteins that were synthesized before the infusion. It is important to point out that depending upon the turnover of a particular protein, ranging from less than 1% per day to over 30% per hour, the incorporation could be extremely fast or slow (46,47). Therefore an extremely sensitive method of detecting low isotopic enrichments is necessary. We initially demonstrated that measurement of low isotopic enrichment in gas chromatography combustion IRMS (48) is accurate, but have since moved to LC/MS/MS because of superior sensitivity and the approach needs only small sample sizes allowing the use of protein spots taken from 2DGE to be used without an extensive derivatization procedure. Because of the relatively high rate of turnover for ApoA-1 (~15% per hour) (47) and the previously mentioned increased oxidative stress (29,30) due to insulin deprivation in T1D individuals, a study was designed to simultaneously measure the age and modification of specific ApoA-1 isoforms (44).

Detailed description of the original method is published (44). Briefly, Seven participants (3 men and 4 women) with T1D for an average of 18.7 years (range 7 – 35 years) were matched to nondiabetic controls. All T1D participants were taking fast acting insulin for at least three days prior to the two trials. In the early morning after an overnight stay in the Clinical Research Unit T1D participants were infused with a continuous infusion of L[ring- $^{13}\text{C}_6$]phenylalanine (Cambridge Isotope Laboratories, Andover, Ma) for eight-hours along with either insulin or saline. Nondiabetic controls received L[ring- $^{13}\text{C}_6$]phenylalanine and saline only. Blood samples were collected every two hours during the trials for isolation of ApoA-1 and its isotopic enrichment as outlined and described in detail in Figure 2.

Future implications – Diabetes and Aging

The results of Jaleel et al (44) clearly demonstrates that the shift in isoelectric points of proteins separated by 2DGE represents modifications to the protein. In addition the nascent ApoA-1 has minimal oxidative damage as opposed to the ApoA-1 synthesized previously and exposed to the environment for a longer period of time. In this study we observed that in T1D *de novo* synthesized ApoA-1 is rapidly oxidized during an 8-hour period of insulin deprivation such that these nascent proteins were rapidly shifted to the location of old proteins. Further, the results of an isotope-based approach and mass spectrometry based analysis of post-translational modifications (44) provide extremely important insights about the mechanism of ApoA-1 damage and poor glycemic control. Specifically, that the higher damage is due to a more rapid rate at which damage is incurred during insulin deprivation in T1D individuals. While the possibility exists that clearance of ApoA-1 may be altered in T1D individuals, it is clear that the primary cause is environmental changes to the plasma

and possibly the liver during insulin deprivation. Therefore potential treatment strategies should address decreasing the harsh environment.

The ability to measure the synthesis rate of individual proteins along with the relative age of the protein is a novel methodology that should be useful for studying other conditions as well. For example, as mentioned previously, aging is likely to be associated with both decreasing protein turnover along with a host of comorbidities. While aerobic and resistance training are known to increase protein synthesis as well as degradation (4). It remains to be determined whether specific exercise modalities have differential effects on synthesis rates of different proteins with different functions. Therefore designing exercise programs to specifically increase the turnover of specific proteins is likely to be an effective therapeutic strategy. There is some evidence in the naked mole rat, where high proteasomal activity is associated with the longest lifespan, greater than 28 years, of any rodent (49) supporting a concept that increased protein degradation and turnover is important for maintaining cellular functions.

In diabetes, both T1D and Type II, where oxidative stress is a hallmark of the disease, therapeutic strategies to reduce the harsh plasma and tissue environment may decrease macrovascular disease rates in this population. Activating the endogenous antioxidant defense system is a strategy that may reduce the effects of oxidative stress. The ability of metformin to activate SKN-1, orthologous to the mammalian Nrf transcription factors (Nrf1 and Nrf2), in *C. elegans* and extend median lifespan in both normal and disrupted insulin signaling animals supports this hypothesis (50). In conclusion, the novel method to identify different ages of proteins that have sustained different degrees of oxidative damage allows investigators to identify *de novo* synthesized protein isoforms and determine whether aged and damaged proteins accumulate in conditions of decreased turnover or increased oxidative stress, thus contributing to various pathologies and dysfunctions.

Acknowledgments

The authors are greatly indebted to the skillful assistance of Maureen Bigelow, Jill Schimke, Katherine Klaus, Dawn Morse, Bushra Ali, Jane Kahl, Dan Jakaitis, Roberta Soderberg, Beth Will, Deborah Sheldon and Melissa Aakre. We are also grateful for support from the National Institutes of Health UL1-RR-024150-01, AG09531 (K.S.N.), R01-DK41973 (K.S.N.), and T32 DK007198 (M.L.J.). Additional support was provided by the Mayo Foundation and the Murdock-Dole Professorship (to K.S.N.).

References

1. Pacy PJ, Bannister PA, Halliday D. Influence of insulin on leucine kinetics in the whole body and across the forearm in post-absorptive insulin dependent diabetic (type 1) patients. *Diabetes Res.* 1991 Dec.18(4):155–162. [PubMed: 1842750]
2. Nair KS, Ford GC, Ekberg K, Fernqvist-Forbes E, Wahren J. Protein dynamics in whole body and in splanchnic and leg tissues in type I diabetic patients. *J. Clin. Invest.* 1995 Jun.95(6):2926–2937. [PubMed: 7769135]
3. Bennet WM, Connacher AA, Jung RT, Stehle P, Rennie MJ. Effects of insulin and amino acids on leg protein turnover in IDDM patients. *Diabetes.* 1991 Apr.40(4):499–508. [PubMed: 2010051]
4. Short KR, Vittone JL, Bigelow ML, Proctor DN, Nair KS. Age and aerobic exercise training effects on whole body and muscle protein metabolism. *Am. J. Physiol. Endocrinol. Metab.* 2004 Jan. 286(1):E92–E101. [PubMed: 14506079]
5. Janssen I, Heymsfield SB, Wang ZM, Ross R. Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *J. Appl. Physiol.* 2000 Jul.89(1):81–88. [PubMed: 10904038]
6. Henderson GC, Nadeau D, Horton ES, Nair KS. Effects of Adiposity and 30 Days of Caloric Restriction Upon Protein Metabolism in Moderately vs. Severely Obese Women. *Obesity.* Nature Publishing Group. 2009 Nov.13:1–8.

7. Glynn EL, Fry CS, Drummond MJ, Dreyer HC, Dhanani S, Volpi E, et al. Muscle protein breakdown has a minor role in the protein anabolic response to essential amino acid and carbohydrate intake following resistance exercise. *Am J Physiol Regul Integr Comp Physiol*. 2010 Aug.299(2):R533–R540. [PubMed: 20519362]
8. Zhang X-J, Chinkes DL, Wolfe RR. Measurement of muscle protein fractional synthesis and breakdown rates from a pulse tracer injection. *Am. J. Physiol. Endocrinol. Metab*. 2002 Oct. 283(4):E753–E764. [PubMed: 12217893]
9. Cuervo AM, Dice JF. Age-related decline in chaperone-mediated autophagy. *J. Biol. Chem*. 2000 Oct.275(40):31505–31513. [PubMed: 10806201]
10. Ferrington DA, Husom AD, Thompson LV. Altered proteasome structure, function, and oxidation in aged muscle. *The FASEB Journal*. 2005 Apr.19(6):644–646.
11. Mai S, Muster B, Bereiter-Hahn J, Jendrach M. Autophagy proteins LC3B, ATG5 and ATG12 participate in quality control after mitochondrial damage and influence lifespan. *Autophagy*. 2012 Jan.8(1):47–62. [PubMed: 22170153]
12. Zhang C, Cuervo AM. Restoration of chaperone-mediated autophagy in aging liver improves cellular maintenance and hepatic function. *Nat Med*. 2008 Sep.14(9):959–965. [PubMed: 18690243]
13. Kumar V, Atherton P, Smith K, Rennie MJ. Human muscle protein synthesis and breakdown during and after exercise. *Journal of Applied Physiology*. 2009 May 26; 106(6):2026–2039. [PubMed: 19164770]
14. Balagopal P, Schimke JC, Ades P, Adey D, Nair KS. Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise. *Am. J. Physiol. Endocrinol. Metab*. 2001 Feb.280(2):E203–E208. [PubMed: 11158921]
15. Irving BA, Robinson MM, Nair KS. Age effect on myocellular remodeling: Response to exercise and nutrition in humans. *Ageing Res. Rev*. 2011 Nov.
16. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature*. 2001 Dec.414(6865):813–820. [PubMed: 11742414]
17. Brownlee M. The Pathobiology of Diabetic Complications: A Unifying Mechanism. *Diabetes*. 2005 Jun.54(6):1615–1625. [PubMed: 15919781]
18. Nyström T. Role of oxidative carbonylation in protein quality control and senescence. *EMBO J*. 2005 Apr.24(7):1311–1317. [PubMed: 15775985]
19. Telci A, Cakatay U, Salman S, Satman I, Sivas A. Oxidative protein damage in early stage Type 1 diabetic patients. *Diabetes Res. Clin. Pract*. 2000 Dec.50(3):213–223. [PubMed: 11106836]
20. Furukawa S. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J. Clin. Invest*. 2004 Dec.114(12):1752–1761. [PubMed: 15599400]
21. de Vries HE, Witte M, Hondius D, Rozemuller AJM, Drukarch B, Hoozemans J, et al. Nrf2-induced antioxidant protection: a promising target to counteract ROS-mediated damage in neurodegenerative disease? *Free Radic. Biol. Med*. 2008 Nov.45(10):1375–1383. [PubMed: 18824091]
22. Jung K-A, Kwak M-K. The Nrf2 System as a Potential Target for the Development of Indirect Antioxidants. *Molecules*. 2010 Jan.15(10):7266–7291. [PubMed: 20966874]
23. Henderson GC, Dhatariya K, Ford GC, Klaus KA, Basu R, Rizza RA, et al. Higher muscle protein synthesis in women than men across the lifespan, and failure of androgen administration to amend age-related decrements. *The FASEB Journal*. 2008 Sep.23(2):631–641.
24. Safdar A, Hamadeh MJ, Kaczor JJ, Raha S, Debeer J, Tarnopolsky MA. Aberrant mitochondrial homeostasis in the skeletal muscle of sedentary older adults. *PLoS ONE*. 2010; 5(5):e10778. [PubMed: 20520725]
25. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science*. 1997 Jun.276(5321):2045–2047. [PubMed: 9197268]
26. Podlisny MB, Lee G, Selkoe DJ. Gene dosage of the amyloid beta precursor protein in Alzheimer's disease. *Science*. 1987 Oct.238(4827):669–671. [PubMed: 2960019]
27. Owen F, Poulter M, Lofthouse R, Collinge J, Crow TJ, Risby D, et al. Insertion in prion protein gene in familial Creutzfeldt-Jakob disease. *Lancet*. 1989 Jan.1(8628):51–52. [PubMed: 2563037]

28. Bateman RJ, Munsell LY, Morris JC, Swarm R, Yarasheski KE, Holtzman DM. Human amyloid- β synthesis and clearance rates as measured in cerebrospinal fluid in vivo. *Nat Med.* 2006 Jun.12(7): 856–861. [PubMed: 16799555]
29. Domínguez C, Ruiz E, Gussinye M, Carrascosa A. Oxidative stress at onset and in early stages of type 1 diabetes in children and adolescents. *Diabetes Care.* 1998 Oct.21(10):1736–1742. [PubMed: 9773740]
30. Martín-Gallán P, Carrascosa A, Gussinye M, Domínguez C. Biomarkers of diabetes-associated oxidative stress and antioxidant status in young diabetic patients with or without subclinical complications. *Free Radic. Biol. Med. Elsevier.* 2003; 34(12):1563–1574.
31. Grattagliano I, Vendemiale G, Boscia F, Micelli-Ferrari T, Cardia L, Altomare E. Oxidative retinal products and ocular damages in diabetic patients. *Free Radic. Biol. Med.* 1998 Aug.25(3):369–372. [PubMed: 9680184]
32. Tabak O, Gelisgen R, Erman H, Erdenen F, Muderrisoglu C, Aral H, et al. Oxidative lipid, protein, and DNA damage as oxidative stress markers in vascular complications of diabetes mellitus. *Clin Invest Med.* 2011; 34(3):E163–E171. [PubMed: 21631993]
33. Klitgaard H, Mantoni M, Schiaffino S, Ausoni S, Gorza L, Laurent-Winter C, et al. Function, morphology and protein expression of ageing skeletal muscle: a cross-sectional study of elderly men with different training backgrounds. *Acta Physiologica Scandinavica.* 1990 Sep.140(1):41–54. [PubMed: 2275404]
34. Turner R, Holman R, Cull C, Stratton I, Matthews D, Frighi V, et al. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet. LANCET LTD.* 1998; 352(9131)
35. Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet.* 2004 Aug.364(9438):937–952. [PubMed: 15364185]
36. Charlton MR, Nair KS. Role of hyperglucagonemia in catabolism associated with type 1 diabetes: effects on leucine metabolism and the resting metabolic rate. *Diabetes.* 1998 Nov.47(11):1748–1756. [PubMed: 9792544]
37. Jaleel A, Klaus KA, Morse DM, Karakelides H, Ward LE, Irving BA, et al. Differential effects of insulin deprivation and systemic insulin treatment on plasma protein synthesis in type 1 diabetic people. *Am. J. Physiol. Endocrinol. Metab. American Physiological Society.* 2009 Oct. 297(4):E889.
38. Du X-L, Edelstein D, Rossetti L, Fantus IG, Goldberg H, Ziyadeh F, et al. Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc. Natl. Acad. Sci. U.S.A. National Academy of Sciences.* 2000 Oct.97(22):12222.
39. Karakelides H, Asmann YW, Bigelow ML, Short KR, Dhatariya K, Coenen-Schimke J, et al. Effect of insulin deprivation on muscle mitochondrial ATP production and gene transcript levels in type 1 diabetic subjects. *Diabetes.* 2007 Nov.56(11):2683–2689. [PubMed: 17660267]
40. Feillet-Coudray C, Rock E, Coudray C, Grzelkowska K, Azais-Braesco V, Dardevet D, et al. Lipid peroxidation and antioxidant status in experimental diabetes. *Clinica Chimica Acta.* 1999 Jun. 284(1):31–43.
41. Lanza, IR.; Zhang, S.; Ward, LE.; Karakelides, H.; Raftery, D.; Nair, KS. Quantitative Metabolomics by 1H-NMR and LC-MS/MS Confirms Altered Metabolic Pathways in Diabetes. In: Wang, Y., editor. *PLoS ONE.* Vol. 5. 2010 May 10. p. e10538
42. White CR, Darley-Usmar V, Berrington WR, McAdams M, Gore JZ, Thompson JA, et al. Circulating plasma xanthine oxidase contributes to vascular dysfunction in hypercholesterolemic rabbits. *Proc. Natl. Acad. Sci. U.S.A.* 1996 Aug.93(16):8745–8749. [PubMed: 8710942]
43. Guzik TJ, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, Pillai R, et al. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. *Circulation.* 2002 Apr 9; 105(14):1656–1662. [PubMed: 11940543]

44. Jaleel A, Henderson GC, Madden B, Klaus K. Identification of De Novo Synthesized and Relatively Older Proteins. *Diabetes*. 2010
45. Mateos-Cáceres PJ, García-Méndez A, López Farré A, Macaya C, Núñez A, Gómez J, et al. Proteomic analysis of plasma from patients during an acute coronary syndrome. *J. Am. Coll. Cardiol.* 2004 Oct.44(8):1578–1583. [PubMed: 15489088]
46. Jaleel A, Short KR, Asmann YW, Klaus KA, Morse DM, Ford GC, et al. In vivo measurement of synthesis rate of individual skeletal muscle mitochondrial proteins. *AJP: Endocrinology and Metabolism*. 2008 Aug.295(5):E1255–E1268.
47. Jaleel A, Nehra V, Persson X-MT, Boirie Y, Bigelow M, Nair KS. In vivo measurement of synthesis rate of multiple plasma proteins in humans. *Am. J. Physiol. Endocrinol. Metab.* 2006 Jul. 291(1):E190–E197. [PubMed: 16449301]
48. Balagopal P, Ford GC, Ebenstein DB, Nadeau DA, Nair KS. Mass Spectrometric Methods for Determination of [13C]Leucine Enrichment in Human Muscle Protein. *Anal. Biochem.* 1996 Jul. 239(1):77–85. [PubMed: 8660628]
49. Pérez VI, Buffenstein R, Masamsetti V, Leonard S, Salmon AB, Mele J, et al. Protein stability and resistance to oxidative stress are determinants of longevity in the longest-living rodent, the naked mole-rat. *Proc. Natl. Acad. Sci. U.S.A. National Academy of Sciences*. 2009 Mar.106(9):3059.
50. Brian Onken MD. Metformin Induces a Dietary Restriction–Like State and the Oxidative Stress Response to Extend *C. elegans* Healthspan via AMPK, LKB1, and SKN-1. *PLoS ONE. Public Library of Science*. 2010; 5(1)

Fig. 1.

The accumulation of damaged and modified proteins in any given protein pool is regulated by both the turnover rate of that protein and the rate of damage. Therefore factors affecting either the rate of protein turnover or rate of damage are likely to affect the quality of the proteome.

Matched peptides shown in **Bold Red**

1	MKAAVLTAV	LFLTGSQARH	FWQQDEPPQS	PWDRVKDLAT	VYVDVLKDSG
51	RDYVSQFEGS	ALGKQLNLKL	LDNWDSVTST	FSKLREQLGP	VTQEFWDNLE
101	KETEGLRQEM	SKDLEEVKAK	VQPYLDDFQK	KWQEEMELYR	QKVEPLRAEL
151	QEGARQKLHE	LQEKLSPLGE	EMRDRARAHV	DALRTHLAPY	SDELQRQLAA
201	RLEALKENG	ARLAEYHAKA	TEHLSTLSEK	AKPAEDLRQ	GLLPVLESFK
251	VSFLSALEEY	TKKLNTQ			

1	MKAAVLTAV	LFLTGSQARH	FWQQDEPPQS	PWDRVKDLAT	VYVDVLKDSG
51	RDYVSQFEGS	ALGKQLNLKL	LDNWDSVTST	FSKLREQLGP	VTQEFWDNLE
101	KETEGLRQEM	SKDLEEVKAK	VQPYLDDFQK	KWQEEMELYR	QKVEPLRAEL
151	QEGARQKLHE	LQEKLSPLGE	EMRDRARAHV	DALRTHLAPY	SDELQRQLAA
201	RLEALKENG	ARLAEYHAKA	TEHLSTLSEK	AKPAEDLRQ	GLLPVLESFK
251	VSFLSALEEY	TKKLNTQ

Fig. 2. Identification of *de novo* synthesized and relatively older proteins. **A:** Amino acid sequence of ApoA-1 spots. Propeptide RHFQQ (in the blue box) for spots D and E, which defines those as ApoA-1 precursors. The propeptide portion was absent in spot-trains A, B, and C, and thus those are mature forms. Red indicates sequence coverage. **B:** Isotopic Enrichment during labeled amino acid infusion was measured in ApoA-1 spot trains isolated from plasma. Spot E had the highest levels of IE, corroborating with propeptide identification

indicating that it was the newest isoform, whereas A was the oldest. MPE, moles percent excess; Hr, hours of tracer infusion. Means $\pm$ SE for n = 7 ND participants. Statistical analysis by ANOVA. Main effect of isoform, $P < 0.0001$; main effect of time, $P < 0.0001$; α Different from 2-h time point, $P < 0.05$; #different from isoform D, $P < 0.05$; *different from isoforms A, B, and C; $P < 0.05$. **C:** Shows tandem mass spectrometry data of deamidation allowing for highly precise and repeatable quantification of protein damage from spots E (newly synthesized) and spots C and B (relatively older isoforms). The red and blue highlight the deamidation of asparagine (N) and glutamine (Q) and oxidation of tryptophan (W), histidine (H), and methionine (M). The amount of deamidation clearly shows that the older spots have more damage ($B > C > E$). **D:** Extent of modification (carbonylation) of spots. **1** and **2** represent the same protein sample run in parallel gels. **1** is visualized by silver stain to identify the spot locations, while **2** is an immunoblot allowing for a crude estimation of carbonylation that was detectable in spots A, B, and C of ApoA-1 as opposed to spots D and E, which are newly synthesized and showed complete absence of oxidative damage/modification. Taken in full, these data show that the 2DGE separation of ApoA-1 isoforms represents modifications to the protein pool based upon the relative age of the protein; with increased irreversible oxidative damage accumulating with the age of the protein. Adapted with permission from (44).