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# Caloric Intake and Alzheimer's Disease

## Experimental Approaches and Therapeutic Implications

*Giulio Maria Pasinetti, Zhong Zhao, Weiping Qin, Lap Ho,  
Yemul Shrishailam, Donal MacGrogan, Wendy Ressmann,  
Nelson Humala, Xunxian Liu, Carmen Romero, Breton Stetka,  
Linghong Chen, Hanna Ksiezak-Reding, Jun Wang*

Neuroinflammation Research Laboratories, Department of Psychiatry, Mount Sinai School of Medicine, New York, N.Y., and Bronx Veterans Affairs Medical Center, Mount Sinai School of Medicine, Bronx, N.Y., USA

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### Abstract

Alzheimer's disease (AD) is a rapidly growing public health concern with potentially devastating effects. Presently, there are no known cures or effective preventive strategies. While genetic factors are relevant in early-onset cases, they appear to play less of a role in late-onset sporadic AD cases, the most common form of AD. Due to the fact that the disease typically strikes very late in life, delaying symptoms could be as good as a cure for many people. For example, it is now widely accepted that if the onset of the disease could be delayed by even 5 years, the incidence could be cut in half. Both clinical and epidemiological evidence suggests that modification of lifestyle factors such as nutrition may prove crucial to AD management given the mounting experimental evidence suggesting that brain cells are remarkably responsive to 'what somebody is doing'. Among other nongenetic factors influencing AD, recent studies strongly support the evidence that caloric intake may play a role in the relative risk for AD clinical dementia. Indeed, the effect of diet in AD has been an area of research that has produced promising results, at least experimentally. Most importantly, as mechanistic pathways are defined and their biochemical functions scrutinized, the evidence supporting a direct link between nutrition and AD neuropathology continues to grow. Our work, as well as that of others, has recently resulted in the development of experimental dietary regimens that might promote, attenuate or even reverse features of AD. Most remarkably, while we found that high caloric intake based on saturated fat promotes AD type  $\beta$ -amyloidosis, conversely we found that dietary restriction based on reduced carbohydrate intake is able to prevent it. This evidence is very exciting and is, in part, consistent with current epidemiological studies suggesting that obesity and diabetes are associated with a >4-fold increased risk of developing AD. The clarification of the mechanisms through which

dietary restriction may beneficially influence AD neuropathology and the eventual discovery of future ‘mimetics’ capable of anti- $\beta$ -amyloidogenic activity will help in the development of ‘lifestyle therapeutic strategies’ in AD and possibly other neurodegenerative disorders.

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The health-related risks associated with obesity are of great public health concern in view of the fact that obesity may causally promote the incidence of a number of chronic degenerative conditions. Some of these conditions, such as Alzheimer’s disease (AD) and Parkinson’s disease, involve the nervous system and are being increasingly linked to issues relating to nutrition. For example, accumulating evidence indicates that certain nutrition-related issues, such as diabetes [1–7], increasing caloric intake [8] or obesity [9, 10], may promote neurodegeneration. In contrast, other nutritional factors, such as dietary restriction [11–14] or consumption of fish oil [15, 16], may beneficially modulate neurodegenerative disorders.

From an economic point of view, neurodegenerative disorders have the potential to become major public health burdens as life expectancy increases. An estimated 4.5 million people have AD in the USA, a number that has doubled since 1980 and is expected to reach as much as 16 million by 2050 [17]. Most importantly, it has been calculated that even delaying the onset of AD for a few years would decrease its prevalence and burden on public health systems [18]. Obesity is also an important health issue when it comes to forecasting future cash flows in the Social Security and Medicare systems. Not only does the likelihood of having comorbidities rise with the degree of obesity, ‘but the prevalence of having 2 or more health conditions’ has been found to increase with weight status [19]. Moreover, in addition to the rather easily quantifiable impact of obesity on morbidity and mortality, being overweight or obese frequently compromises individuals’ quality of life [20].

Thus, it is of concern that at the beginning of the twenty-first century, the fraction of Americans considered to be obese had reached ‘epidemic’ levels, according to a study published in the *Journal of the American Medical Association* [21]. This study, which was carried out between 1991 and 1998, observed a steady increase in weight in all states of the union, in both sexes, across age groups, races and educational levels, and occurred regardless of smoking status. It found that obesity had increased from 12.0% in 1991 to 17.9% in 1998. Likewise, ‘national survey data show that between 1976–80 and 1988–94 the age-adjusted prevalence of obesity increased by 8 percentage points, from 14.5 to 22.5%, in the US adult population ages 20–74’ [22]. This translates into increases in mean body mass index (BMI) and in the prevalence of overweight and obesity for US adults and children.

In an effort to establish a basis to define what a 'healthy weight' is, the Dietary Guidelines for Americans [6th ed., 2005; <http://www.healthierus.gov/dietaryguidelines/>] addresses issues of weight maintenance and weight loss by making long-term changes in physical activity and eating behavior. The health consequences of overweight and obesity are related to adverse health conditions such as diabetes, coronary heart disease and hypertension. One glaring fact about the recommended range of healthy weights in the Dietary Guidelines is that close to more than half of the adult population, in particular half of adult males, have been above that range at least since 1960. A discussion of the pros and cons of this latest edition shows how complex and elusive the subject of optimal weight can be [23]. Of interest is that leptin, a hormone important in energy homeostasis and food intake regulation, has been singled out in the guidelines as a metabolic indicator influencing food intake. Because levels of leptin rise when fat stores are high, leptin may play a role in public health monitoring of adiposity in the future [24].

Of particular interest to this review article is the accumulating evidence pointing to a relationship between obesity and dementia later in life. For example, Whitmer et al. [25] have recently reported an analysis of prospective data from a multiethnic population-based cohort obtained with the objective of evaluating a possible association between obesity in middle age, as measured by BMI and skinfold thickness, and risk of dementia later in life. Dementia was diagnosed in 713 (6.9%; in a cohort of 10,276 people) of the participants. Obese people (BMI  $\geq 30$ ) had a 35% greater risk of dementia compared with those of normal weight (BMI 18.6–24.9). The authors concluded that obesity in middle age increases the risk of future dementia independently of comorbid conditions. This evidence is very interesting especially in view of the recent studies suggesting that certain cardiovascular risk factors (e.g. diet) may be significant contributors to an increased risk of vascular-related dementia [1]. A large body of evidence indicates that cardiovascular risk factors, e.g. certain dietary ones, may also increase the relative risk of AD and clinical dementia even when vascular dementia cases are excluded from the analysis [26, 27]. Thus, it may be the case that additional 'nonvascular' events associated with certain cardiovascular risk factors may be involved in the increased risk for AD. Most interestingly, we also note that recent evidence suggests that type 2 diabetes may also be associated with an increased risk of developing AD and may affect cognitive systems differentially [2]. Thus, it is possible that potential risk factors associated with certain dietary regimens accepted as cardiovascular risk factors for vascular dementia may also independently contribute to the development and progression of AD. Further exploration of this phenomenon in AD and AD model systems will provide critical direction for future studies investigating mechanisms involved in the potentiation of AD neuropathology and

possibly future therapeutic applications based on dietary modifications. However, as discussed below, dietary regimens including dietary restriction (DR) and weight reduction programs in neurodegenerative disorders such as AD can be a complex endeavor because they should be made on the basis of combined evidence from different sources such as (1) epidemiological studies, (2) experimental models and ultimately (and most importantly) (3) from controlled clinical studies.

Based on these considerations, this review article will first discuss recent evidence indicating (1) the beneficial role of dietary regimens in health and disease and (2) recent experimental evidence suggesting that diet and possibly the control of caloric intake may beneficially influence AD and possibly other neurodegenerative disorders. Finally, based on this evidence, this review will identify potential therapeutic scenarios for eventual future interventions.

### **Dietary Implications in the Onset and Progression of Clinical Alzheimer's Disease**

The possibility that reactive oxygen species are a factor in the neuronal damage seen in AD has led to examine how antioxidants in foods, or as vitamin supplements in the form of tocopherol (vitamin E), ascorbic acid (vitamin C) and carotenes, can affect AD. Although the results appear promising in some cases, the data on the value of antioxidant supplementation remain inconclusive. Likewise, deficiencies in folate, vitamin B<sub>12</sub> and vitamin B<sub>6</sub> lead to high concentrations of homocysteine in the brain through different pathways. This has led to study the possible link between homocysteine and the development of AD and Parkinson's disease [28]. Although epidemiological studies have implicated high concentrations of homocysteine in the brain in neuronal degeneration [11, 28], the data relating folate and vitamin B<sub>12</sub> and B<sub>6</sub> supplementation as palliatives to the cognitive decline seen in AD are inconsistent [28].

However, Lim et al. [15] have recently used a transgenic mouse model of AD-type neuropathology to evaluate the impact of n-3 fatty acid docosahexaenoic acid (DHA) in AD-type amyloid neuropathology. They found that DHA-enriched diets significantly reduced total AD-type amyloid neuropathology by >70% when compared with low-DHA or control chow diets. Dietary DHA also decreased  $\beta$ -amyloid (A $\beta$ ) 1-42 levels below those seen with control chow. The results suggest that DHA could be protective against AD-type amyloid deposition in the brain and eventually prevent downstream neurodegenerative conditions.

Studies relating fat and fish intake to the risk for AD and cognitive decline have failed to establish a firm causal relationship. Despite the fact that there are

no controlled clinical studies to support dietary recommendations, in an indirect way, a diet low in saturated and *trans*-fatty acids and high in monounsaturated, polyunsaturated and fish-related fats can be assumed to be beneficial in the prevention of cognitive decline and AD by means of promoting a viable vascular system [29]. Furthermore, dietary fats may also influence AD through lipid metabolism, insulin resistance [30], high concentrations of circulating insulin [3], oxidation [31] and the amyloid cascade hypothesis [32, 33]. In addition, *APOE*, the gene associated with sporadic AD, is related to lipid metabolism and modulates cholesterol concentrations in response to intake from fats [34, 35]. People with the *APOE*  $\epsilon$ 4 allele, who are at high risk of AD, normally have high levels of cholesterol in the blood.

In addition to nutrients, alcohol intake has also been suggested as a risk factor for AD. This is supported by evidence that ethanol consumption might lead to oxidative brain damage in rat models [36]. Paradoxically, accumulating epidemiological evidence indicates that moderate consumption of alcohol in the form of red wine may actually lower the risk of cerebrovascular disease in older adults [37]. A study by Goldberg et al. [38] sponsored by the American Heart Association has found that consumption of alcohol-containing beverages (e.g. wine) actually has cardiovascular and cognitive benefits. However, numerous other studies relating alcohol to AD have yielded mixed results. A case has been made for the elderly to consume red wine in moderate quantities because of its high contents of antioxidants, such as flavonoids, which may not be found in other alcoholic beverages [4, 39, 40]. But, in and of itself, alcohol consumption carries the potential for abuse and addiction.

Observational studies on diet and disease may incur errors in the measurement of nutrients, a caveat that has been clarified by Luchsinger and Mayeux [4]. If the measurement error is not related to outcome, it will underestimate true associations. Also, the long latency period of AD may be the consequence of lifelong exposure to a number of factors that are difficult to isolate and analyze in their true context. Moreover, the validity of basing clinical decisions about individual patients on data from randomized trials remains to be settled [41]. So far, it appears unlikely that trials can address all questions regarding diet and AD, given the nature of AD as a chronic disease with a latency period. It would be difficult to conduct trials long and large enough to observe results.

Luchsinger and Mayeux [4] also touched on the concept that nutritional supplements alone (e.g. carotenoids) may not be as effective as the whole foods in which they may be found (fruits and vegetables) such that the interactions of nutrients within foods, or patterns of diet, is what may actually be of benefit. Recent findings by Gardner et al. [41] appear to corroborate their viewpoint. According to their findings, plant-based diets may be superior to low-fat diets even if the two diets are identical in total fat, protein, carbohydrate and cholesterol

content. These authors noted that national dietary guidelines have probably underestimated the potential low-density lipoprotein cholesterol-lowering effect of certain diets. In a randomized clinical trial, they set out to contrast plasma lipid responses to two low-fat diet patterns. They found that a plant-based low-fat diet reduces levels of low-density lipoprotein twice as much as a low-fat diet based on prepackaged foods. Such plant-based diets may provide an effective alternative to cholesterol-lowering drugs like statins [41].

Although there is enough evidence suggesting that dietary modification, such as low calorie intake, may prevent AD and other age-related neurodegenerative disorders, malnutrition in the elderly remains a concern. Hence, dietary recommendations may need to be made on the basis of comorbidities such as type 2 diabetes, cardiovascular disease and osteoarthritis.

### **Alzheimer's Disease and Nutrition**

While genetic factors are highly relevant in early-onset AD cases, their significance diminishes in late-onset sporadic AD cases, the most common form of AD [17]. Nongenetic factors, including modifiable lifestyle dietary regimens, are receiving great attention in AD, especially because of recent epidemiological studies indicating that caloric intake may influence the relative risk for AD clinical dementia. Dietary factors have been an area of research that has produced promising results, at least experimentally. Most importantly, the evidence supporting a direct link between nutrition and AD amyloid neuropathology discussed below [12, 13] continues to grow, as the mechanistic pathways are defined and their biochemical functions scrutinized.

AD is a progressive neurodegenerative disorder marked by loss of memory, cognition and behavioral stability [17]. AD is defined pathologically by extracellular neuritic plaques comprised of fibrillar deposits of  $\beta$ -amyloid ( $A\beta$ ) and neurofibrillary tangles comprised of paired helical filaments of hyperphosphorylated tau. Current therapies for AD, such as cholinesterase inhibitors, treat the symptoms but do not modify the progression of the disease. The etiology of AD is unclear, and data from familial AD mutations strongly support the 'amyloid cascade hypothesis' of AD, i.e. that neurodegeneration in AD is initiated by the formation of neurotoxic  $A\beta$  aggregates, and all familial AD mutations increase levels of  $A\beta$  peptide or the density of  $A\beta$  deposits [17].

Current therapeutic strategies to treat AD are aimed at preventing the formation of amyloidogenic  $A\beta$  peptides [17]. For this reason, the 'amyloidogenic'  $\beta$ - and  $\gamma$ -secretase activities necessary for the generation of amyloidogenic  $A\beta$  peptides have become central targets for development of therapeutic reagents in AD [17]. However, it has been difficult to find safe, selective  $\beta$ - and  $\gamma$ -secretase

inhibitors, mainly because of the influence of these inhibitors on other cellular substrates whose processing is vital [17]. Ongoing studies, discussed below, in our laboratory suggest that DR regimens based on low-carbohydrate content may beneficially influence AD-type neuropathology through the promotion of ‘nonamyloidogenic’ processing of amyloid precursor protein (APP) via the promotion of  $\alpha$ -secretase activities. In addition, since the  $\alpha$ -secretase cleavage of APP is known to involve the release of a soluble neuroprotective form of APP (sAPP- $\alpha$ ; also found in our studies), it is possible that DR, while promoting the nonamyloidogenic pathway in the brain, may also promote brain repair activities as a consequence of sAPP- $\alpha$  neurotrophic function [42].

As discussed below, there is increasing consensus that the production and accumulation of A $\beta$  peptides is central to the pathogenesis of AD. The continual search for ways to manage if not reverse AD neuropathology has led to ongoing efforts to elucidate its underlying causes and possible treatments. The likely link between A $\beta$  peptide aggregation and AD pathology emphasizes the need for a better understanding of the mechanisms through which dietary regimens may influence A $\beta$  production.

### **Diabetogenic Dietary Regimens Resulting in Insulin Resistance Coincide with Promotion of Alzheimer’s Disease Amyloid Neuropathology**

Abnormalities in insulin metabolism associated with type 2 diabetes resistance are among the central factors thought to influence the onset of AD by promoting the synthesis and/or interference of A $\beta$  degradation [5, 6, 43–45]. For example, there is *in vitro* evidence indicating that insulin itself may significantly promote the generation of extracellular amyloidogenic A $\beta$  peptides through mechanisms that involve the acceleration of APP/A $\beta$  trafficking from the *trans*-Golgi network, a major cellular site for A $\beta$  generation, to the plasma membrane [43]. While this evidence tentatively suggests that abnormal carbohydrate metabolism might play an important role in AD through mechanisms that involve A $\beta$  peptide generation, experimental studies also suggest that insulin resistance may promote AD amyloid neuropathology in the Tg2586 mouse model of AD amyloid neuropathology, possibly by limiting A $\beta$  degradation via competition with insulin for degradation by insulin-degrading enzyme (IDE) [46], a zinc-metallopeptidase that preferentially cleaves proteins with a propensity to form  $\beta$ -pleated sheet-rich amyloid fibrils [47], such as monomeric A $\beta$  peptides [46].

While the role of insulin in AD has received major attention with respect to its potential role in amyloid neuropathology, recent evidence also suggests a role

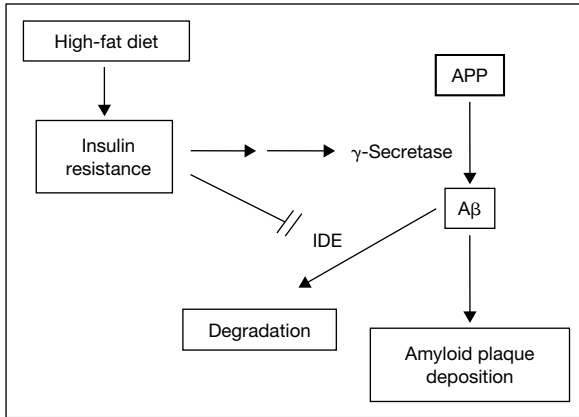
for insulin in normal memory function, supporting the hypothesis that insulin affects many mechanisms related to neuronal activity and cognitive function by itself. Of interest to us is the fact that chronic hyperinsulinemia and insulin resistance, or reduced insulin effectiveness, may exert a negative influence on memory [5]. For example, Hoyer [44] proposed that low concentrations in circulating insulin in the central nervous system, together with reduced expression of IR and subsequent altered downstream signaling AD, would ultimately lead to reduced levels of acetylcholine with a corresponding decrease in cerebral blood flow.

Based on this evidence and the fact that type 2 diabetes appears to be associated with an increased relative risk for AD [5, 6, 44, 45], we have recently explored the role of experimental type 2 diabetes in the Tg2576 AD mouse model [6]. We found that a diabetogenic diet resulting in elevated circulating levels of insulin coincided with promoted amyloidogenic  $A\beta_{1-40}$  and  $A\beta_{1-42}$  peptide generation and amyloid plaque burden in the brain of Tg2576 mice that corresponded with increased  $\gamma$ -secretase activities and decreased IDE activities. Moreover, the increased AD-type amyloid neuropathology also coincided with increased and impaired spatial memory functions assessed by performance in a spatial water maze task [6]. Further exploration of the apparent interrelationship of insulin resistance to brain amyloidosis revealed a functional decrease in IR-mediated signal transduction in the brain, as suggested by decreased IR  $\beta$ -subunit (IR- $\beta$ ) Y<sup>1162/1163</sup> autophosphorylation and reduced phosphatidylinositol 3-kinase/pS<sup>473</sup>-AKT/protein kinase B in these same brain regions [6]. Results from this study strongly suggested that one mechanism through which diet-induced insulin resistance in Tg2576 mice can significantly promote AD-type amyloidosis in the brain is by reducing IR signaling, resulting in elevation of  $\gamma$ -secretase activities. The studies also suggested that type 2 diabetes may further contribute to AD amyloid neuropathology attenuating degradation of  $A\beta$  peptides through pathways associated with IDEs (fig. 1).

Collectively, these findings indicate that clinical AD is a result of early life as well as later life risk factors, and that genetic predisposition to the disease may modify the constellation of predictors.

### **Dietary Restriction Based on a Low-Carbohydrate Diet**

As discussed above, a fundamental problem of AD neuropathology is the aberrant generation of amyloidogenic  $A\beta$  amyloid peptides in the brain that lead to an abnormal deposition of the neuritic plaques that are a landmark in AD. Although evidence supports a potential neuroprotective role for DR in neurodegeneration, until recently there was no information as to whether reduced caloric intake could attenuate AD neuropathology. Findings of recent



**Fig. 1.** Role of insulin resistance in AD-type neuropathology.

prospective studies indicate that increasing caloric intake may be a risk factor for AD [45, 48, 49]. Because of this evidence and the epidemiological evidence indicating that DR may influence the risk for AD [8, 45], we have continued to explore if a clinically applicable weight reduction/DR regimen based on an approximately 30% reduced carbohydrate content could (1) attenuate AD neuropathology and (2) decrease preexisting amyloid neuritic neuropathology (e.g. a reduction in plaque size), eventually resulting in recovery of amyloid-associated neuritic dystrophy as a function of time in the same strain of Tg2576 mice fed a low-carbohydrate/DR diet. Based on these considerations, we tested the hypothesis that low-carbohydrate/DR may be a beneficial intervention in AD through mechanisms that prevent A $\beta$  generation and neuritic plaque deposition in the brain using a mouse model of AD type amyloidosis [50]. The aim of these studies was to test the hypothesis that DR may beneficially influence AD through mechanisms that prevent the development of amyloidosis associated with AD.

To test this hypothesis, 3-month-old Tg2576 mice, which develop AD type amyloid neuropathology by 8–10 months of age [50], were fed for 9 months with a daily low-carbohydrate diet resulting in a 30% lower caloric intake compared to that consumed by age- and gender-matched control Tg2576 mice fed ad libitum (AL) with a standard laboratory rodent diet. Nutrient composition in the DR diet was achieved by selectively reducing the carbohydrate content of the diet while consumption of protein, fat, cholesterol, vitamins and minerals was identical to that of AL fed Tg2576 mice [12]. This dietary regimen resulted in body weight stabilization over the 9-month study period among DR Tg2576

mice relative to the AL fed group, coinciding with an approximately 3-fold lower ependymal fat pad weight and improved glucose tolerance response as determined by an intraperitoneal glucose tolerance test. These physiological adaptations in the DR Tg2576 mice relative to AL fed controls are consistent with clinical evidence that low-carbohydrate DR considerably improves abnormal glucose control and obesity [9, 51–53], which are risk factors not only for diabetes but also for AD [7, 54].

### **Weight Reduction Dietary Restriction Results in Decreased Alzheimer's Disease-Type Amyloid Neuropathology in Tg2576 Mice**

When Tg2576 mice were examined for AD-type neuropathology at 12 months of age, we found that 9-month DR treatment almost completely prevented cortical and hippocampal AD-type amyloid plaque development [12] relative to animals in the AL fed group. Consistent with this evidence, we noted commensurately lower concentrations of amyloidogenic A $\beta$ <sub>1–40</sub> and A $\beta$ <sub>1–42</sub> peptides in the neocortex and hippocampus as evaluated by ELISA assay, relative to AL fed controls [12]. No detectable change in total full-length APP level was noted in either brain region of DR versus AL fed Tg2576 mice [12].

To further evaluate the antiamyloidogenic role of DR in the brain of Tg2576 mice, we explored APP processing and A $\beta$  peptide generation using immunoprecipitation (IP) and mass spectrometry (MS). Consistent with the aforementioned ELISA evidence, using 4G8 antibody for A $\beta$  IP, we confirmed decreased levels of A $\beta$ <sub>1–40</sub> and A $\beta$ <sub>1–42</sub> in the same neocortical samples we used for the A $\beta$  ELISA assay [12]. In addition, a relative proportional reduction in A $\beta$ <sub>1–37</sub>, A $\beta$ <sub>1–38</sub> and A $\beta$ <sub>1–39</sub> peptide content was also observed in the neocortex of the DR group compared to the AL fed control group. This evidence, together with our observation that the concentration of the approximately 7-kDa carboxy-terminal fragment (CTF)  $\gamma$  cleavage product of APP, an index of  $\gamma$ -secretase activity, was unchanged in the neocortex of the DR group relative to AL fed controls, suggested the possibility that  $\gamma$ -secretase activity was not involved in the DR-associated antiamyloidogenic activity.

To further identify A $\beta$  carboxy-terminal peptide fragments that would have been otherwise undetected in the 4G8 IP-MS studies, we used 6E10 antibody in additional A $\beta$  IP-MS studies. Consistent with the 4G8 IP-MS spectra, we noted decreased levels of A $\beta$ <sub>1–40</sub> and A $\beta$ <sub>1–42</sub> as well as A $\beta$ <sub>1–37</sub>, A $\beta$ <sub>1–38</sub> and A $\beta$ <sub>1–39</sub> peptide in the DR group relative to AL fed control animals. In addition, we found a major elevation in A $\beta$ <sub>1–16</sub> peptide fragment concentration in the neocortex of the DR group that was not detected in the AL fed controls.

Because  $\alpha$ -secretase can cleave APP, eventually resulting in the generation of A $\beta$  CTFs ending at the AA residue leucine 16 of A $\beta$  [42], we continued to explore the role of DR in  $\alpha$ -secretase activity in the brain.

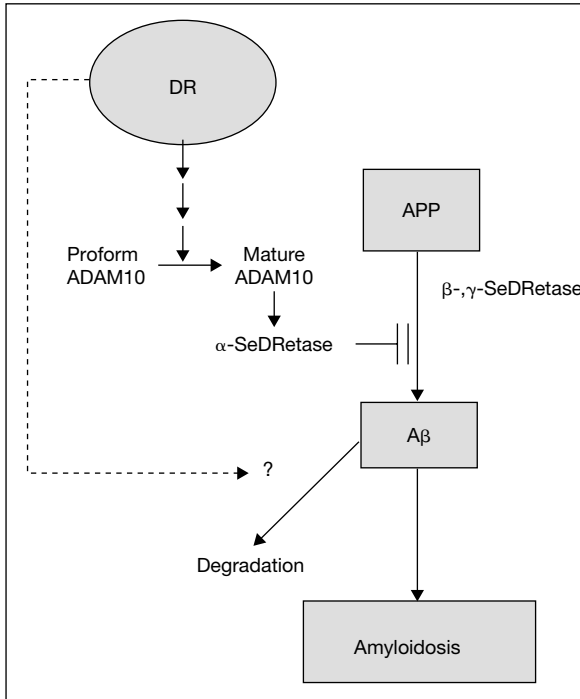
Cleavage of APP by  $\alpha$ -secretase releases the amino-terminal extracellular domain known as sAPP- $\alpha$  domain coincidental with elevation in membrane-bound  $\alpha$ -secretase-cleaved APP CTF- $\alpha$ . We therefore explored the regulation of sAPP- $\alpha$  and CTF- $\alpha$  cleavage products of APP in the brain as indices of  $\alpha$ -secretase activity in response to DR. Interestingly, we found that DR in Tg2576 mice resulted in a >2-fold elevation in the concentration of neocortical sAPP- $\alpha$  and membrane-associated CTF- $\alpha$  relative to AL fed control Tg2576 mice. The increase in CTF- $\alpha$  was somewhat less, about 1.6-fold, presumably because of further cleavage of CTF- $\alpha$  by  $\gamma$ -secretase. Compared with the CTF- $\alpha$  fragment, the abundance of CTF- $\beta$  signal was at the limit of detection in the neocortex of both DR and AL fed Tg2576 mice, preventing reliable quantification [12].

### **Weight Reduction Dietary Restriction Diet May Influence $\alpha$ -Secretase Activity in the Brain in Part by Selectively Promoting the Generation of Mature and Catalytically Active ADAM10 Species**

In light of recent evidence indicating that the proteinase ADAM10 (a disintegrin and metalloproteinase) may act as an  $\alpha$ -secretase [42], we continued to explore the regulation of ADAM10 expression in the brains of Tg2576 mice in response to DR, relative to AL fed controls. Both mature (62-kDa) and proform (90-kDa) ADAM10 species were detected in the neocortex of the AL fed control animals, confirming previous evidence [12]. The 62-kDa mature ADAM10 protein species is known to act as an  $\alpha$ -secretase in vitro and to cleave A $\beta$ -derived peptides at leucine 16 [12, 42]. Excitingly, we found that the DR diet regimen resulted in a 30% elevation of neocortical mature ADAM10 species concentration, coinciding with a commensurate elevation in neocortical  $\alpha$ -secretase activity, compared to AL fed control mice.

As shown in figure 2, this evidence supports the hypothesis that low-carbohydrate DR may prevent AD-type amyloid neuropathology through mechanisms that influence  $\alpha$ -secretase activity in the brain, possibly by promoting the generation of mature, catalytically active ADAM10 species. Since  $\alpha$ -secretase proteolysis of the APP sequence within the A $\beta$  peptide would preclude the generation of amyloidogenic A $\beta$  peptides, our studies suggest that DR may provide an attractive anti-amyloidogenic strategy by promoting  $\alpha$ -secretase activity in the brain.

In addition to promoting  $\alpha$ -secretase activity, we found that DR led to a small, but significant elevation of IDE content in the brain of Tg2576 mice. The



**Fig. 2.** Role of DR in the prevention of AD-type amyloid neuropathology.

role of IDE in A $\beta$  degradation was demonstrated by recent studies showing that mice deficient for IDE exhibit increased cerebral accumulation of endogenous A $\beta$  peptides. Thus, it is possible that the attenuation of A $\beta$  burden in the brain of DR Tg2576 mice might also derive from enhanced IDE-mediated clearance of A $\beta$  peptides in addition to the promotion of the nonamyloidogenic  $\alpha$ -secretase cleavage of APP [5, 12, 46]. In view of a recent study from Patel et al. [13] reporting the A $\beta$ -lowering efficacy of a DR diet in additional mouse models of AD amyloid neuropathology, it is likely that the beneficial effect of a ‘low-carbohydrate/DR’ diet on A $\beta$  neuropathology and cognitive function in the Tg2576 AD mouse model [12] may reflect the impact of DR, per se. However, it is possible that the low carbohydrate content in the ‘low-carbohydrate/DR’ dietary regimen may promote additional disease-modifying activities.

As discussed above, current strategies to treat AD are aimed at preventing formation of amyloidogenic A $\beta$  peptides. Therefore,  $\beta$ - and  $\gamma$ -secretases that generate A $\beta$  peptides by sequential cleavage of the APP or degrade released A $\beta$  peptides are obvious and central targets for the development of therapeutic

reagents. Our evidence showing that DR may positively influence  $\alpha$ -secretase, possibly through mechanisms that may involve the generation of mature, catalytically active ADAM10 species in the brain, might prove in the future the basis of potential novel preventative measure aimed at delaying the onset of AD neuropathology. In addition, since  $\alpha$ -secretase cleavage of APP releases sAPP- $\alpha$ , which is well known for its neuroprotective properties, our study tentatively suggests that promoting a low-carbohydrate DR dietary regimen may also result in increased brain repair activities as a consequence of sAPP- $\alpha$  neurotrophic function. However, we cannot exclude the possibility that DR might also influence other mechanisms, eventually resulting in decreased amyloid deposition in the brain by promoting  $\alpha$ -site cleavage of APP or degradation of released A $\beta$  by other proteases such as plasmin and neprilysin, respectively. In addition to promoting A $\beta$ -lowering activity, DR may also benefit AD through mechanisms not directly related to generation and/or degradation of A $\beta$  of peptides. In particular, DR is known to reduce inflammation [55] and oxidative stress [56], two of the major contributing factors in AD-type neurodegeneration [57, 58]. Therefore, it would not be unexpected that DR may beneficially modulate the onset and/or progression of neuropathology and neurodegeneration in AD through multiple mechanisms. Thus, the relationship between caloric intake and AD could have important implications in the prevention and/or therapy of AD [17].

DR is well known to improve insulin sensitivity responses, especially in insulin resistance conditions such as type 2 diabetes [59, 60]. Based on the observation that diet-induced insulin resistance promotes the generation of A $\beta$  peptides, it would not be unexpected that A $\beta$ -lowering activity of DR may be related to promotion of insulin sensitivity responses. However, evidence indicates that insulin resistance and DR may have independent impacts on A $\beta$  generation and that diabetogenic and DR diet appears to activate independent signal transduction pathways ultimately influencing APP processing and generation of A $\beta$  peptides [6, 12]. While a diabetogenic diet induces A $\beta$  generation by promotion of the AKT-GSK pathway [6], ongoing studies showed that DR may reduce A $\beta$  generation by activating  $\alpha$ -secretase activity (perhaps activation of ADAM10 activity), in part, via promotion of the MAPK-PKC signaling pathway. Further studies in our laboratory are presently aiming to better understand whether DR in obese-diabetic Tg2576 mice may reverse AD-type amyloidogenic activities via modulation of these specific signal transduction pathways.

## Conclusion

Study findings support existing epidemiological evidence indicating that caloric intake is positively associated with the increased incidence of AD and

raises the possibility that changes in dietary regimens may be used in future preventative measures aimed at delaying the onset of AD amyloid neuropathology. Investigations in experimental mouse models of AD neuropathology, such as ours, are of great potential benefit in terms of public health because they provide insights into possible interventions to prevent or ameliorate conditions associated with those over 65 years of age in the USA. This is the age group with the highest incidence of excess weight, obesity and diabetes, and it is the largest group associated with the highest risk to develop AD dementia.

We want to point out, however, that decisions on diet recommendations in AD can be a complex endeavor because they should be made on the basis of combined evidence from different sources such as (1) experimental models, (2) prospective epidemiological studies and ultimately (3) controlled clinical studies. While we believe that the ultimate evidence to support such recommendations should come from controlled clinical trial studies, we are also aware of the potential limitation of this approach. For example, we point out that, in view of the chronic nature of AD dementia with a relatively long latency period, it may be difficult to execute appropriate clinical studies for enough time and in large enough samples to draw accurate and repeatable conclusions.

However, despite these limitations, we believe the recent prospective studies showing that increased caloric intake is a risk for AD [8] and the recent observation that that DR [12, 13] in AD mouse models may beneficially influence AD neuropathology provide strong impetus to ascertain the validity of a DR diet in AD patients.

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Giulio Maria Pasinetti, MD, PhD  
Neuroinflammation Research Laboratories, Department of Psychiatry  
Mount Sinai School of Medicine, 1 Gustave L. Levy Place, Box 1230  
New York, NY 10029–6574 (USA)  
Tel. +1 212 659 8716, Fax +1 212 876 9042, E-Mail giulio.pasinetti@mssm.edu