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Amyloid fibrils: potential food safety implications

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ABSTRACT

The demonstration of oral Amyloid-A (AA) fibril transmissibility has raised food safety questions about the consumption of amyloidotic viscera. In a presumed prion-like mechanism, amyloid fibrils have been shown to trigger and accelerate the development of AA amyloidosis in rodent models. The finding of amyloid fibrils in edible avian and mammalian food animal tissues, combined with the inability of cooking temperatures to eliminate their amyloidogenic potential, has led to concerns that products such as pâté de foie gras may activate a reactive systemic amyloidosis in susceptible consumers. Given the ability of amyloid fibrils to cross-seed the formation of chemically heterologous fibrils, the speculative etiologic role of dietary amyloid in other disease processes involving amyloid formation such as Alzheimer's disease and Type II Diabetes is also discussed.

The 1997 Nobel Prize in Medicine was awarded for the etiological understanding of a novel class of diseases that appeared at the same time spontaneous, heritable and infectious (Nobelprize.org, 1997). Prion diseases appear to arise from a post-translational change in conformation of normally monomeric, soluble, proteinase K-sensitive and largely D-helical proteins (Prion Protein Cellular, or PrP^c) into E-sheet-rich prions (proteinaceous infectious particles, so-called Prion Protein Scrapie (PrP^{Sc})) which may form insoluble protease-resistant aggregates (Collins et al., 2004). This transformation may occur spontaneously, as might be the case with sporadic Creutzfeldt–Jakob Disease (CJD); as a result of a germ line mutation of the prion protein gene, as seen in familial CJD; or via an infectious mode of transmission, as seen in kuru, for example, an orally acquired human prion disease epidemic propagated by the consumption of dead relatives among the Fore linguistic group of Papua New Guinea (Johnson, 2005).

There are two models of PrP^c to PrP^{Sc} transformation, one envisaged as a heterodimeric catalytic chain reaction and the other a nucleated polymerisation cascade in which a fibril-like nidus of PrP^{Sc} is elongated by the conformational conversion and addition of PrP^c monomers. Fragments of such aggregates may then seed further prion replication (Collins et al., 2004). The high degree of homology between cattle and primate prion proteins may explain the ability of bovine prions to misfold human PrP^c (Choi et al., 2006), resulting in the invariably fatal neurodegenerative disease variant CJD (CJD) among some genetically predisposed individuals consuming tissue from cattle infected with Bovine Spongiform Encephalopathy (BSE) (Collee, Bradley and Liberski, 2006).

Prion diseases constitute a subset of amyloidoses, a broader class of disorders characterised by secondary structure misfolding of a heterologous array of normally soluble proteins into insoluble fibrils sharing a common cross-E core structure. These fibrils tend to accumulate extracellularly, generating amyloid deposits that may disrupt tissue structure and function. PrP^c is but 1 of 26 different precursor proteins known capable of forming amyloid *in vivo* (Yan et al., 2007). There is growing evidence that amyloid fibrils other than prions may be a potential source of foodborne contagion as well.

1 Amyloid-A fibril infectivity

Amyloid-A (AA) amyloidosis, also called reactive systemic amyloidosis (previously, 'secondary' systemic amyloidosis), involves the deposition of amyloid derived from Serum Amyloid-A protein (SAA), an acute phase reactant. Levels of circulating SAA can increase a thousand-fold in reaction to injury or infection (Röcken and Shakespeare, 2002), returning to baseline at the conclusion of the inflammatory response. Chronic insults or autoimmune disorders, however, can lead to persistently high SAA concentrations. In a subset of patients with prolonged SAA elevation, fibrils composed of E-sheet-folded N-terminal fragments of SAA precipitate out of solution and become lodged in tissues. Pieces of elongating fibrils may then break off and enucleate further amyloid deposits throughout the body (Lundmark et al., 2002).

The development of AA amyloidosis can thus be split into two phases. The protracted SAA elevation caused by sustained inflammation is described as the preamyloid phase which, in humans, can last for years without amyloid deposition. The second, the amyloid phase, is marked by the build-up of amyloid triggered by the generation of the first nidus of fibrillar network to initiate the conversion cascade. On autopsy, kilograms of this amorphous material may be found permeating organs. Median survival is 4–10 years after diagnosis (Obici et al., 2005), though this grave prognosis can be forestalled with heart, liver, or kidney transplants of the most affected organs (Pepys, 2001).

AA amyloidosis can be reproduced in laboratory animal models via injections with irritating substances such as turpentine (Molteni and Mombelloni, 1964). Subjection to repeated inescapable electric shocks can also eventually produce the disease (Hall, Cross and Hall, 1960). In the 1960s, researchers established that the duration of the pre-amyloid phase could be dramatically shortened in chronically inflamed mice by injecting them with extracts of the diseased organs of mice dying with AA amyloidosis. An 'amyloid-enhancing factor' that accelerated the process was posited in amyloid-ridden organs. Subsequent research into this mysterious factor identified it unequivocally as the AA fibril itself (Lundmark et al., 2002).

Intravenous injection of less than a picogram of AA fibrils can rapidly seed the formation of widespread amyloid deposits throughout the bodies of chronically inflamed animals (Zhang et al., 2006). Magy et al. (2003) was able to demonstrate this process *in vitro*. Seeds of AA fibrils bound to fibroblast monolayers were shown to act as a sink for SAA, leading to the formation of amyloid networks radiating from the fibril precipitates. In light of the recognition of vCJD secondary to BSE, Elliott-Bryant and Cathcart (1998) fed amyloidosis-diseased organs to susceptible mice and were the first to demonstrate prion-like oral transmission.

2 Foie gras

Reports dating back to 1933 offer accounts of spontaneous amyloidosis in ducks caged in laboratories and on farms (Cowan and Johnson, 1970b). Investigating the appearance of the disease in birds at zoos, Cowan and Johnson (1970a) concluded the appearance of the disease in ducks was primarily related to the chronic stress of confinement. They showed that AA amyloidosis could be reliably reproduced in

healthy ducks via simple overcrowding. In the flock with the highest stocking density, spontaneous deaths from amyloidosis began to occur at six months of age.

Foie gras, French for 'fatty liver,' is typically produced by force-feeding ducks until their steatotic livers swell to 6–10 times their normal weight (Scientific Committee on Animal Health and Animal Welfare (SCAHAW), 1998a). Stressors associated with foie gras production identified by the European Commission's Scientific Committee on Animal Health and Animal Welfare (1998b) include pain and injury from feeding tube insertion, fear and stress during capture and handling, gait abnormalities due to liver distention and pathological hepatic function. Given the susceptibility of ducks under chronic stress to spontaneous amyloidosis and the demonstration of AA fibril oral transmissibility, the amyloidogenic potential of foie gras came under investigation.

Solomon et al. (2007) found green birefringent congophilic areas by polarizing microscopy in several commercial sources of foie gras, including pâté de foie gras, which immunostained with specific anti-AA antibodies, providing immunohistochemical evidence of AA amyloid deposits in marketed foie gras products. Electron microscopy corroborated the ultrastructural amyloid features, and AA composition was confirmed chemically by tandem mass spectrometry and amino acid sequencing.

Foie gras extracts were then intravenously injected into mice transgenically modified to express chronically high SAA levels. Within eight weeks, virtually all of the treated mice, but none of the control animals, developed amyloid deposits. Similar results were obtained using the conventional murine model of AA amyloidosis, wild-type mice exposed to an inflammatory stimulus. Within three weeks, amyloid was found in eight of ten such mice injected with foie gras extract and none of the inflamed controls (Solomon et al., 2007).

Oral transmission was also demonstrated through the administration of foie gras extracts by gavage into eight of the transgenic mice. Five of the animals went on to develop amyloid deposits in virtually all organs examined. The amyloidosis accelerating effect of foie gras was reduced, but not eliminated, by first cooking the product as specified by the supplier. The investigators conclude:

“Given our experimental findings...it would seem prudent for children and adults with Rheumatoid Arthritis (RA) or other diseases who are at risk for this disorder [AA amyloidosis] to avoid foods that may be contaminated with AA fibrils” (Solomon et al., 2007).

3 Potentially Susceptible Populations

Like prions, AA fibrils have been shown to cross the gut barrier (Gruys, 2004) and trigger disease. The oral “infectious dose” of AA fibrils, at less than a microgram, is comparable with the infectivity of prions (Zhang et al., 2006), and species barriers can be surmounted (Gruys, 2004). Presumably because of similarities in structure and composition, AA fibrils also exhibit similar resistance to physical and chemical decontamination methods. Treatment with cooking (Solomon et al., 2007), freezing/thawing, and disinfectants such as formalin and 2N NaOH may not abolish AA fibril infectivity. Zhang et al. (2006) found that autoclaving for three hours likewise did not guarantee inactivation. They conclude:

“These results suggested strongly that amyloid diseases could be transmitted like prion diseases under certain conditions.”

One important difference between AA fibril and prion infectivity is that the development of AA amyloidosis appears to necessarily require elevated levels of the precursor protein (Soto, Estrada and Castilla, 2006). At baseline low concentrations, SAA circulates with its amyloidogenic N-terminus tightly bound to high-density lipoprotein. Only when serum levels rise may SAA become free to interact with AA-derived fibril seeds (Lundmark et al., 2002).

Conditions associated with elevated SAA levels may include chronic infections, such as tuberculosis, leprosy, malaria, and osteomyelitis (Gertz and Kyle, 1991), as well as noninfectious chronic inflammatory disease (Pepys, 2006), such as RA, juvenile RA, other inflammatory arthritides like ankylosing spondylitis (Röcken and Shakespeare, 2002), and psoriatic arthritis (Gertz and Kyle, 1991), Crohn's disease (Pepys, 2006), ulcerative colitis (Röcken and Shakespeare, 2002), lupus, bronchiectasis (Gertz and Kyle, 1991), sarcoidosis (Röcken and Shakespeare, 2002), familial Mediterranean fever, other hereditary periodic fever syndromes (Pepys, 2006), and certain malignancies such as Hodgkin's disease, mesothelioma (Röcken and Shakespeare, 2002), and renal cell carcinoma (Gertz and Kyle, 1991).

In the West, with chronic infections such as leprosy in decline, RA now accounts for more than 60% of AA amyloidosis cases. The average onset of clinical amyloidosis after RA diagnosis is reported may be 19 years (Hazenbergh and Rijswijk, 2000). Autopsy studies indicate that as many as 21% of RA patients eventually develop the disease (Suzuki et al., 1994). Of Crohn's patients, 0.5–6% may also develop this potentially fatal complication (Lovat et al, 1997). In 5% of cases of AA amyloidosis, no specific cause for the SAA elevation can be found (Röcken and Shakespeare, 2002).

Any condition involving chronic inflammation can result in sustained SAA overproduction, and perhaps 10% of individuals with persistently elevated SAA levels may eventually develop AA amyloidosis (Pepys, 2006). In rare cases, it can appear within a year of clinically apparent inflammatory disease, but typically takes years to develop (Röcken and Shakespeare, 2002). The concern raised by Solomon et al. is that foie gras consumption by individuals with high SAA levels may trigger and/or accelerate this process (Solomon et al., 2007). Further findings, though, suggest that a broader segment of the population may be at risk.

Lundmark et al. (2002) repeated experiments showing that normal, healthy mice exposed orally or parenterally to AA fibrils do not develop amyloidosis, whereas those additionally receiving a concurrent inflammatory stimulus develop pronounced disease within days. But, what if healthy mice are exposed to AA fibrils and then inflammation is induced at some later date? Might the AA fibrils remain lodged inside tissues, priming the recipients for rapid induction of AA amyloid should SAA levels rise in the future? Indeed, Lundmark et al. (2002) found that even months after fibril exposure, an inflammatory stimulus could rapidly induce AA amyloidosis to the same extent as concurrent inflammation and fibril injection.

The longest interval between exposure and inflammation studied was 180 days (Lundmark et al., 2002), nearly one-quarter of the animals' natural lifespan (Löhrke, Hesse and Goerttler, 1984). This suggests that consumers of foie gras may be at increased risk for AA amyloidosis should they develop an inflammatory disorder potentially years after consumption. So, in addition to those with active disease, the principal investigator of the foie gras study has suggested that

“[p]erhaps people with a family history of...RA or other amyloid-associated diseases should avoid consuming foie gras and other foods that may be contaminated” (University of Tennessee Graduate School of Medicine, 2007).

The induction of amyloid deposition in mice nursed by amyloid fibril-injected mothers underscores this concern (Korenaga et al., 2006).

Although people with a family history of RA do appear at higher risk for the disease, based on twin studies, the genetic component may be minor (Gregersen, 1998). Given the inability to accurately prognosticate who will develop many of the broad range of disorders that can lead to prolonged inflammation, it may be prudent to generally avoid ingesting amyloid-diseased organs (Tojo et al., 2005).

4 Cross-seeding

Solomon et al. (2007) suggest that foie gras consumption may also be particularly hazardous to those prone to Alzheimer's disease or Type II Diabetes (T2D). This concern is based on experimental evidence that chemically heterologous fibrils can each seed the formation of the other, a process known as cross-seeding.

Though amyloid fibril formation may be a generic property of polypeptide chains (Dobson, 1999), in vivo, only 26 different proteins are known to form fibrils naturally (Yan et al., 2007). Since amyloidoses are classified by the amassing protein, 26 different types of amyloidosis have been described. Irrespective of protein sequence homology or native conformation, all amyloid fibrils seem to share a common protofilament substructure of stacked β -sheets (Sunde et al., 1997).

This structural similarity may explain not only why human AA fibrils can demonstrably seed AA amyloidosis in mice, but also why four other human amyloidoses – all involving different proteins (amyloid- λ , amyloid-TTR, amyloid- β_2 M and β -Synuclein) can also cross-seed AA amyloidosis in mice (Fu et al., 2004). Cui et al. (2002), for example, orally administered semi-purified human light chain-derived (AA) amyloid fibrils to mice, waited three weeks before triggering an inflammatory state, and found that AA amyloid deposition was rapidly induced in 11 of 15 treated mice. None of the control mice receiving either the fibrils or inflammatory stimulus alone developed detectable amyloid deposition.

Based on this cross-seeding principle, might any fibril organised as a well-ordered repetitive helical array of long axis-parallel β -sheets function as a shape-transforming scaffold and nucleate similar fibrillar cascades? Spider silk, for example, is composed of fibrils of β -pleated sheets, as is the silk of the silkworm (*Bombyx mori*). *Escherichia coli* has convergently evolved curli, analogously structured fibrillar adhesive fimbriae. Indeed, Kisilevsky et al. showed in a murine model that injection of a few micrograms of a silk fibril preparation could dramatically accelerate the formation and deposition of AA amyloid (Kisilevsky, Lemieux, Boudreau, Yang and Fraser, 1999).

This leads to some provocative conclusions. Lundmark et al. (2005) speculated that

“[t]his mechanism may be of great importance for the understanding of the pathogenesis of human AA amyloidosis and, perhaps, other forms of amyloidosis. Exposure (by ingestion or inhalation) to naturally occurring fibrils like silk, Sup35, or curli may bring seeds that start a nucleation process in predisposed individuals with persistently high SAA production.”

They note a case control study of occupational risk factors among genetically predisposed individuals for clinical amyloidosis that found an odds ratio of 5.4 for dressmakers (presumably exposed to silk dust) (Hardell et al, 1995).

AA amyloidosis only accounts for a fraction of the amyloidoses diagnosed in Westerners; most cases of systemic amyloidosis are caused by amyloid proteins other than AA. AO amyloidosis is the most common non-AA systemic manifestation (previously known as 'primary' amyloidosis), an invariably fatal disease caused by the build-up of antibody proteins or protein fragments created in excessive amounts by plasma cell tumors. After approximately 5–7 years on hemodialysis, patients develop deposits of E2M amyloid, a protein normally cleared by the kidneys. Eventually, most dialysis recipients suffer from it (Pepys, 2001). The prevalence of the mutation transthyretin Val122Ile among African–Americans may be as high as 3.9%, suggesting approximately one million African–Americans may be at significant risk for congestive heart failure due to this familial amyloidosis (Jacobson et al., 1997). Senile systematic amyloidosis affects nearly everyone by age 90. Though usually asymptomatic, massive cardiac involvement can lead to heart failure (Pepys, 2001).

Though it may be reasonable to advise those with a long history of hemodialysis, for example, to abstain from eating products containing AA fibrils (Tojo et al., 2005), the proscription for those with a family history of Alzheimer's or diabetes assumes an etiologic role for amyloid in these disease processes. Amyloid deposits do tend to accumulate in the brains of Alzheimer's victims and the pancreatic islet cells of T2D, but it is not yet clear whether this represents cause or effect (Pepys, 2006).

The accumulation of amyloid β ($A\beta$) has been described alternately as both 'an instrumental, if not sole, culprit for causing [Alzheimer's] disease' and, at the same time, more of an 'innocent bystander' (Rottkamp et al., 2002). $A\beta$ amyloidosis can be experimentally transmitted to primates via intracerebral injection of Alzheimer's brain homogenate (Baker et al., 1994). This has been accepted as evidence that Alzheimer's disease is transmissible (Riek, 2006), but unlike the unambiguous clinical manifestation of prion transmission (death), the more subtle and variable presentations of a neurodegenerative disease like Alzheimer's are more difficult to diagnose in non-human animals (Walker et al., 2006). The role played by amyloid- β in Alzheimer's disease remains uncertain, so even if anseriform AA fibrils in foie gras could reach the human brain and cross-seed $A\beta$ deposition, for example, this would not necessarily manifest as Alzheimer's disease. It would be useful to know if feeding foie gras to transgenic (Tg2576) mice expressing human amyloid- β proteins could accelerate $A\beta$ amyloid deposition as is the case when such 'humanised' mice are intracerebrally injected with dilutions of Alzheimer's brain homogenate (Walker et al., 2002).

The role of amyloid in the development of T2D is even more speculative. While the build-up of Islet Amyloid Polypeptide (IAPP) in insulin-secreting cells is a hallmark of a substantial proportion of T2D (Hull et al., 2004), the role amyloid IAPP plays in the disease remains unclear. Some 'consider T2D to be a form of islet Alzheimer disease' (Prentki and Nolan, 2006) and even suggest that one of the reasons diabetics have higher rates of Alzheimer's is that pancreatic amyloid fibrils may be cross-seeding amyloid- β in their own brains (Yan et al., 2007), though IAPP fails to seed the formation of $A\beta$ (1–40) fibrils in vitro (O'Nuallain et al., 2004). Amyloid deposits (including $A\beta$ and AA) have also been found in arterial atherosclerotic plaques, but the role they play, if any, is likewise unknown (Howlett and Moore, 2006), hindering efforts to understanding the extent of the potential risk associated with dietary amyloid exposure.

5 Other Dietary Sources of Amyloid Fibrils

SAA is considered the major vertebrate acute-phase reactant. Evolutionarily, SAA, like PrP^c, appears highly conserved and has been found in every vertebrate species studied to date (Uhlar and Whitehead, 1999). Just as bovine prions fed to mice can trigger a murine spongiform encephalopathy, so too can

bovine AA fibrils fed to mice trigger AA amyloidosis, even weeks after exposure. 'Thus,' Cui et al. (2002) conclude,

"the results of our present study, in which oral ingestion of amyloid fibrils extracted from different species caused amyloid deposition, may be important in understanding the etiology of AA amyloidogenesis in humans."

AA amyloidosis occurs in a wide variety of wild as well as domesticated animals, including chickens, cattle, dogs, goats, horses, sheep and, rarely, cats and pigs (Ménsua et al., 2003). Tojo et al. (2005) found a 'disturbingly high' incidence of AA amyloidosis in slaughtered beef cattle (5%) and conclude that people with chronic inflammatory diseases 'need to avoid' ingesting foods that may possibly contain amyloid fibrils.

A significant fraction of meat-type 'broiler' chickens may be chronically stressed in production (European Commission, Scientific Committee on Animal Health and Animal Welfare, 2000), but their 6–7 week production is likely not enough time to develop amyloidosis. AA amyloidosis has been found in broiler breeder parent stock, though, as well as egg-laying hens. Amyloidosis is becoming an increasing clinical problem in egg-laying hens with up to 20–30% of commercial flocks in several European countries being affected (Landman, 1999). Though the inflammatory stimulus in these cases was primarily infection with *Enterococcus faecalis*, which is present in the US flocks (Hayes et al., 2003), the white leghorn breed more commonly used in the USA is resistant to amyloidosis formation (Landman, 1999).

The amyloid deposits in chickens tend only to accumulate in articular cartilage (Ovelgönne et al., 2001). Although hepatic amyloid infiltration was been found in a layer flock stressed by chronic respiratory infection (Shibatani et al, 1984) and one can experimentally induce amyloidosis in chickens systemically, these birds tend only to localise deposits in their joints, as opposed to ducks which accrue amyloid throughout their visceral organs (Landman et al., 1996; Landman, 1999), AA amyloidosis has been reported in the joints of 61% of chickens found lame on egg farms in Europe (Landman et al., 1996).

Commercial layers and broiler breeders are typically killed at the end of their productive lives. Approximately half of 'spent' hens are slaughtered for human consumption and the other half rendered for products such as animal feed or pet food (Gregory, 2004). The extrusion of spent hens into mechanically separated meat, a paste used in jerky snacks (Minimus, 2008) and fast-food chicken nuggets (Wikipedia, 2008), and their use to make chicken broth (Farkaš et al, 1997) and commercial flavoring base (Sangtherapitikul, Chen and Chen, 2005), may result in joint amyloid contamination of consumer product. The likely inability of the rendering process to eliminate infectivity presents further questions regarding agricultural or veterinary risks.

SAA is highly conserved between fish and humans (Lashuel, 2008), and aging Pacific salmon undergo a rapid senescence with accompanying AE amyloid build-up in their brains (Maldonado, Jones and Norris, 2002), but apparently only one report of systemic amyloidosis in fish appears in the literature and the muscles did not seem affected (Mashima, Cornish and Lewbart, 1997). Liver involvement raised the possibility that a product such as cod liver oil could potentially be contaminated, but protein fractions should largely be purified out of fish oil preparations. To date, foie gras is the only food product shown to accelerate amyloid development (University of Tennessee Graduate School of Medicine, 2007). It is not known whether foie gras consumption leads to an increase in amyloid-related disease rates (University of Tennessee Graduate School of Medicine, 2007). Though undercooked duck liver consumption may cause toxocarasis (Hoffmeister et al, 2007) or toxoplasmosis (Bártová et al, 2004), there are apparently

no published epidemiological studies involving foie gras. There appear few data on dietary amyloidosis risk factors in general (Simms, Prout and Cohen, 1994).

A striking contrast has been noted between the detection rates of AA amyloidosis triggered by leprosy in the West versus India, Africa and Japan (Williams et al., 1965). Whereas approximately 50% of the US cases have shown evidence of amyloidosis on autopsy, for example, a study of 1,222 leprosy cases in India failed to uncover a single example, even though some patients had been suffering for decades with the disease. Gupta and Panda (1980) report:

“Consumption of a mainly vegetarian diet in our population and that of meat in Western population has been suggested to be the probable cause of the difference of amyloidosis observed in the two groups of people.”

Based on this and other leprosy studies implicating meat consumption (Williams et al., 1965), Elliott-Bryant and Cathcart (1998) speculate

“dietary modification may be of therapeutic potential in preventing amyloid fibril formation.”

The Adventist Health Study found that those eating meat appeared to have three times the risk of developing dementia compared to long-time vegetarians (Giem, Beeson and Fraser, 1993), but this is likely confounded by vascular factors (van Duijn, 1996), just as studies linking meat consumption and T2D are confounded by obesity (Vang et al, 2008). The current body of epidemiological data is insufficient to address the issue of amyloid tissue food safety.

6 Conclusions

Using amyloid joint disorders of chickens as an example, Gruys et al. (2005) have suggested that amyloid deposits in the tissues of food animals could have ‘tremendous food safety implications.’ The oral transmissibility data, they concluded, indicate ‘that like prions, this pathological material should be banned for risk groups of consumers.’ The amount of foie gras orally dosed by Solomon et al., however, was the equivalent of feeding a person 1.6–1.7 kg of pâté de foie gras over a five-day period (Raloff, 2007). Although, intravenously, a femtomolar dose of a purified AA fibril preparation (≈ 0.015 ng) has been shown to be amyloidogenic (Lundmark et al., 2002), the oral AA-enhancing dose has yet to be determined, though Zhang et al. did induce amyloidosis in a susceptible murine model with the oral administration of 1 μ g of purified mouse senile amyloid (apolipoprotein A-II) fibrils (Zhang et al., 2006).

Additional research is necessary to quantify the risk, but transenteral time-delayed cross-species amyloid cross-seeding has been experimentally demonstrated. Accordingly, consumers of AA fibril-containing foods such as foie gras arguably risk accelerating a variety of systemic amyloidoses should amyloid precursor protein levels subsequently raise due to conditions such as neoplasm, inflammation, or chronic hemodialysis.

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