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Review Article

Arachidonic Acid and Platelet-Activating Factor as Central Players in the Metabolic Syndrome. Lessons from the Use of the *Ginkgo Biloba* Extract

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Abstract

Diabetes mellitus is a chronic disease with worldwide prevalence and the toll of the disease is substantially increased because it is commonly associated with several chronic disorders, collectively termed the metabolic syndrome. There is continuing efforts to identify the biochemical compound/s linking all of the disorders. I have hypothesized that arachidonic acid and platelet-activating factor, lipids found in the plasma membrane, are central players and have used *Ginkgo biloba* extract to test this hypothesis. The standardized *Ginkgo biloba* leaf extract contains approximately 24% flavonoid glycosides, to which the free radical scavenging properties have been ascribed, and 6% terpene lactones which is thought to be responsible for platelet aggregation inhibit and increase blood flow. This review summarizes the characteristics of the metabolic syndrome and results of more than two decades of clinical studies involving the ingestion of 120 mg of *Ginkgo biloba* extract daily, as a single dose for 3 months in open label or randomized double-blind placebo control trials. It was found that *Ginkgo biloba* extract: (1) decreased *in vivo* platelet reactivity, as seen by decreased plasma thromboxane B_2 and urinary excretion of the thromboxane B_2 metabolites, and *in vitro* reduction of arachidonic acid stimulated platelet thromboxane A_2 production. The later was accompanied by reduced synthesis of malondialdehyde, a reactive oxygen species, in both non-diabetic and diabetic subjects. Importantly it was discovered that Ginkgo inhibited platelet aggregation mediated predominantly by collagen, and to a lesser degree, by platelet-activating factor, (2) stimulated pancreatic β -cell insulin production in both non-diabetic and type 2 diabetic subjects, especially in diabetic subjects with pancreatic exhaustion and (3) did not increase whole body insulin resistance. Taken together, it seems that cell membrane perturbation resulting in the release and metabolism of arachidonic acid and platelet-activating factor might be the underlying cause in the development of several metabolic defects commonly associated with diabetes and the use *Ginkgo biloba* extract allowed us to verify several aspects of the metabolic syndrome.

Keywords: *Ginkgo Biloba; Platelets; Pancreas; Arachidonic Acid; Platelet-Activating Factor*

Introduction

Ginkgo biloba extract as a dietary supplement

The Dietary Supplement and Health and Education Act of 1994 passed by the 103rd Congress of the United States legalized easy access to dietary supplements, without the prior rigorous scientific testing required for orthodox medications as required by the United State Food and Drug Administration (FDA) [1]. Herbal products, as dietary supplements, account for a substantial portion of the complementary and integrated health market. Unlike conventional medicines, research designed to determine the constituent ingredients, their mechanisms of action, efficacy and overall safety are often conducted after rather than before human consumption. The *Ginkgo biloba* tree, originally found mostly in Asia and once thought to be extinct, now lines several streets of major cities in the United States and around the world. *Ginkgo biloba* has essentially remained unchanged compared to its fossils dating back to the early Jurassic period, making it the oldest living tree species, and aptly termed a "living fossil". It has had a very long history as a folk remedy dating back thousands of years and hundreds of studies have been conducted to verify its medicinal value, including its use as a remedy for delaying progression

of senile dementia and Alzheimer's disease, reducing anti-depressant effects associate with sexual dysfunction, and increasing cerebral and cardiovascular blood flow [2-6]. The standardized extract preparation of the *Ginkgo biloba* leaf contains approximately 24% flavonoid glycosides, 6% terpene lactones (ginkgolides and bilobalide), and relatively minor content of ginkgolic acid [7,8]. Free radical scavenging properties have been ascribed to the flavonoids, consisting predominantly of kaempferol, myricetin, and quercetin [9,10] while its ability to inhibit platelet aggregation and increase blood flow has been attributed to the terpenoid fraction, consisting of predominantly the ginkgolides A and B. Ginkgolide B is the most potent in inhibiting platelet aggregation by inhibition of platelet-activating factor (1-O alkyl-2-acetyl-sn-glycero-3-phosphorylcholine, PAF) *in vitro* [6].

The Insulin Resistance (or Metabolic) Syndrome

Diabetes mellitus type 2 (T2DM) is a chronic disease that develops over time when decreasing whole body insulin resistance initially leads to compensatory increased activity of the pancreatic β cells leading to hyperinsulinemia. For a while this may produce no significant changes in fasting blood glucose. Increasing insulin resistance may cause progression first, into a state of impaired fasting glucose and eventually into overt T2DM with hyperglycemia when the compensatory hyperinsulinemia is increase glucose uptake. The resulting hyperinsulinemia becomes the hallmark not only of diabetes but also a key feature of a host of metabolic diseases associated with diabetes termed the metabolic syndrome [11-16].

The term metabolic syndrome, as it is currently used includes the following metabolic defects: hyperinsulinemia, obesity, hypertension, dyslipidemia, hypercoagulation, platelet hypersensitivity, as illustrated in Figure 1. Briefly, results of the San Antonio Heart Study showed that these disease states usually occurred in some combination of three or more disorders [12]. Obesity is a state of increased insulin resistance and is characterized by decreased insulin clearance, increased hepatic glucose production, increased VLDL secretion, increased production of pro-inflammatory factors, and increased production of thrombotic factors [16,17]. It is this impaired insulin sensitivity in the peripheral cells that causes the pancreatic β cells to increase compensatory insulin secretion. Hypertension is more prevalent in the diabetic population than in non-diabetics and is also associated with obesity and a common finding in hypertension as well as T2DM and obesity is dyslipidemia and hyperinsulinemia [16]. People with T2DM have a two- to four-fold increase in the risk of dying from the complications of cardiovascular disease, with atherosclerosis and vascular thrombosis being major contributors and platelet dysfunction contributing significantly to the atherogenic process [13-17]. A major hypothesis relating to the development of the atherogenetic process involves the interaction of platelets with an injured blood vessel with exposed collagen fibers in the subendothelial space and the formation of a clot matrix, the stability of which depends on the balance between arachidonic metabolites - Thromboxane A_2 (TXA_2) and prostacyclin (PGI_2). TXA_2 promotes platelet aggregation and eventually thrombus formation whereas PGI_2, produced principally by the vascular endothelial cells, is an inhibitor of platelet aggregation and thus promotes the disruption of the thrombus plug [17,18]. Dyslipidemia is characterized by increased plasma concentrations of triglycerides and low-density lipoprotein (LDL) cholesterol, overproduction of very low density lipoprotein (VLDL) cholesterol and decreased levels of HDL cholesterol. There is evidence that high levels of cholesterol can play a significant role in the progression of glomerular injury and cholesterol lowering drugs effectively lowering the progression of the renal dysfunction. Kidney damage is a common occurrence in T2DM and proteinuria is a very sensitive predictor of renal damage [19-23]. Type 2 diabetes mellitus also involves low-grade systemic inflammation with pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α) and IL-6 being also common occurrences in obesity [24-26]. TNF-α can inhibit insulin signaling in the liver, increase triglycerides and free fatty acid release from adipose tissues that increase hepatic lipogenesis contributing to development of insulin resistance, while IL-6 appears to be able to impair insulin signaling resulting in impaired insulin sensitivity in hepatocytes.

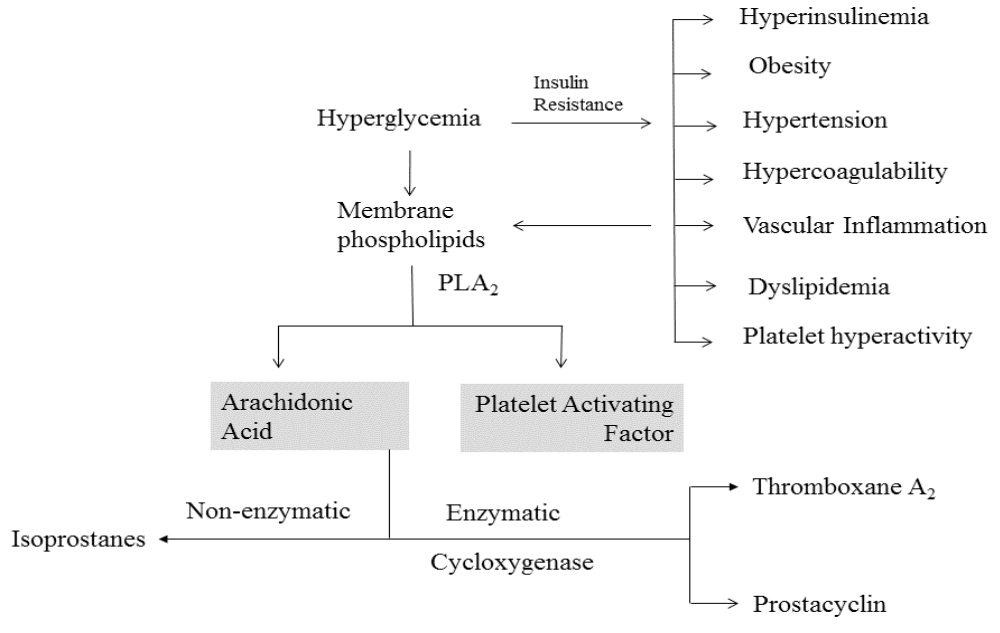

Figure 1: *Metabolic disturbances commonly associated with the metabolic syndrome also affect the production of arachidonic acid and platelet-activating factor.*

Membrane Phospholipids - Arachidonic Acid and Platelet Activating Factor

Type 2 diabetes creates a state of decreased plasma membrane fluidity that may affect phospholipid modeling which may result in enhanced platelet aggregation, and impaired vasodilator response that can lead to the development of hypertension and eventually kidney damage. Perturbation of the plasma membrane leads to the production of two principal phospholipids: arachidonic acid and platelet-activating factor (1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine, PAF). Arachidonic acid is an essential polyunsaturated omega 6 fatty acid that is stored in the plasma membrane and may be released after phospholipase A_2 (PLA_2)-catalyzed hydrolysis. Phospholipase A_2 exists in several isoforms, including the secretory PLA_2, cytosolic PLA_2, and the calcium independent PLA_2 [27-29].

Arachidonic acid appears to be involved in the regulation of inflammation in several tissues including the pancreatic β cell, regulation of blood pressure, and homeostatic functions [30-33]. Arachidonic acid metabolites can be either pro-inflammatory, promoting chemotaxis and production of cytokines, or playing anti-inflammatory roles. They may regulate pancreatic β cell function directly through promoting insulin secretion or indirectly through inflammatory mediators that can alter insulin secretion, signaling, and probably contribute to tissue damage. An essential arachidonic acid metabolic pathway, among many, involves the cyclooxygenase (COX) enzyme and metabolites of arachidonic acid can be implicated in the regulation of blood pressure as vasoconstrictors or vasodilators through this pathway. Cyclooxygenase-1 (COX-1) activity leads to production of prostaglandins that regulate cell membrane activity, the only isoenzyme present in platelets and is responsible for the formation of TXA_2 [18]. The increased intracellular TXA_2 causes the release of cellular Ca^{2+} which modulates changes in the cytoskeleton and platelet shape change leading to platelet aggregation [34]. Increased platelet COX-1-mediated TXA_2 synthesis, platelet aggregation and vasoconstriction may increase the risk of thrombosis, especially in diabetes [35]. COX-2 may be constitutively expressed but is also inducible by various inflammatory and proliferative stimuli, forming pro-inflammatory prostanoids and hence the popular use of COX-2 inhibitors such as Celebrex® and non-steroidal anti-inflammatory drugs (NSAID) for treatment of several inflammatory conditions [36]. In the endothelial cell, COX-2-mediated PGI_2 production leads to vasodilation [37] and could essentially counteract the action of COX-1-induced platelet reactivity and vasoconstriction. Cyclooxygenase-3 (COX-3) is the most recently identified isoform and is thought to also play a role in inflammation [38].

The release of arachidonic acid is accompanied by the release of PAF, which is involved in many important biological responses such as asthma, systemic anaphylaxis, and those that are related to the metabolic syndrome are illustrated in Figure 2. stimulation of neutrophils, macrophages and platelets when responding to a specific stimulus [39,40]. While some cells release most of the PAF that they produce (e.g. monocytes), PAF remains cell-associated in others, such as the endothelial cells and in the glomeruli, where, by mediating changes in cell-shape and cytoskeletal structures, it increases vascular and glomerular permeability which can be reversed using PAF receptors antagonists in animal models [41,42]. Intravenous injection of PAF also reduces blood pressure in a variety of animals [43]. However, PAF is metabolically unstable being rapidly hydrolyzed by plasma acetylhydrolase (EC 3.1.1.48), to the biologically inactive lyso-PAF (1-O-alkyl-2-hydroxy-sn-glycero-3-phosphocholine) [44]. Interestingly, plasma acetylhydrolase is preferentially associated with low density lipoprotein (LDL) cholesterol particles which are found to be elevated in essential hypertension [45], atherosclerosis and coronary artery disease [46], in T2DM and in non-diabetic obese subjects [47]. It seemed conceivable that increased kidney production of PAF could lead to increased vascular permeability and extravasation of proteins – the marker for renal damage. Indeed, we found urinary PAF excretion to be increased approximately 20-fold in T2DM patients compared to non-diabetic subjects [48].

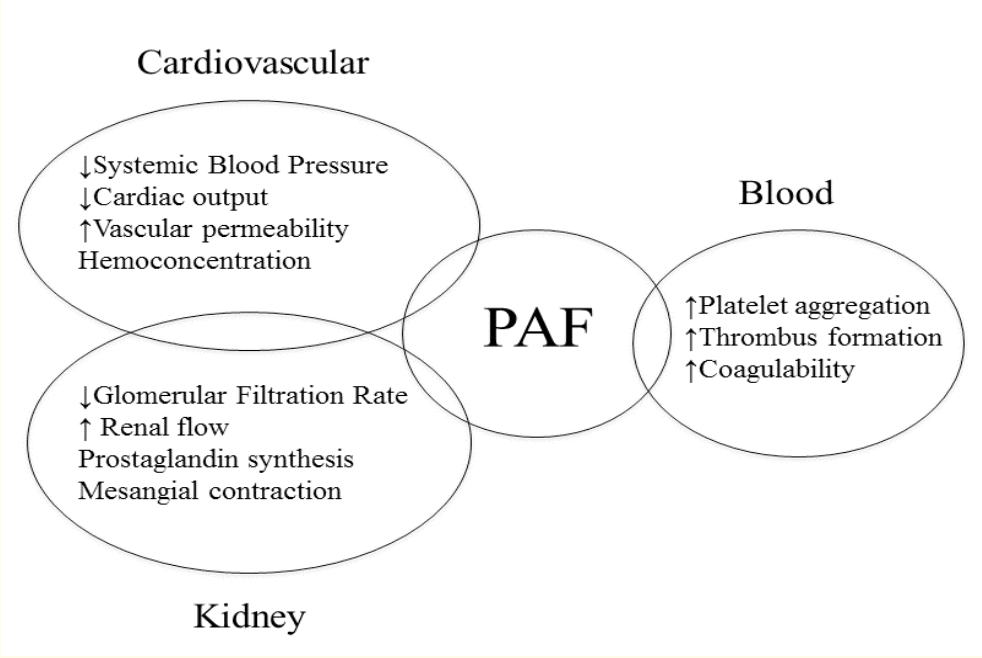

Figure 2: *Platelet-activating factor (PAF) is associated with several organ systems commonly assisted with the metabolic syndrome.*

Taken together, it appeared that many of the disease states that are a part of the metabolic syndrome are connected to metabolic processes that intertwine with the metabolism of arachidonic acid and platelet-activating factor from the plasma membrane of a variety of cells. After establishing that diabetics (a) produced and excreted excessive amount of PAF and (b) had increased plasma acetylhydrolase activity, the first step was to determine if the use a PAF receptor antagonist could reverse these conditions. The use of the dietary *Ginkgo biloba* extract was ideal because it contained confirmed potent PAF receptor antagonists ginkgolide B, and was easily obtainable over-the-counter in the United States.

The *Ginkgo biloba* studies

Ginkgo biloba study designs

All the studies were approved by the Institutional Review Board and written, informed, voluntary consent was obtained from all subjects before the studies began. All subjects were screened, using laboratory tests including comprehensive metabolic panel, and complete blood counts, before being enrolled into the studies. Body weights for all individuals enrolled had been stable for at least 3 months prior to study. None of the participants was on a dietary or exercise program for weight reduction. Potential subjects with significant medical problems (major cardiovascular, hepatic and other endocrine disease) other than elevated total cholesterol, as determined by routine medical history, physical examination, blood and urine tests were excluded. A pregnancy test was performed in all women of reproductive potential, and pregnant or nursing women were excluded. Subjects with anemia [Hb < 12 g/dL (female) or < 13 g/dL (male)] and diabetic subjects on insulin were also excluded. The volunteers underwent the standard 2-hour oral glucose tolerance test (OGTT) at the start of the experiments to verify glucose tolerance and provide quantitative information about pancreatic β -cell secretion using established procedures [49]. Blood samples were collected during the basal state for the measurement of the comprehensive metabolic panel - liver function, the total lipid profile, fibrinogen, the integrity of the intrinsic (prothrombin time, PT) and extrinsic (partial thromboplastin time, PTT) pathways of the coagulation system. Venous blood (30 ml) was collected platelet aggregation studies. For three months, the subjects ingested 120 mg (as a single dose) of clearly labeled 50:1 standardized *Ginkgo biloba* extract, with guaranteed potency of 24% Ginkgo flavone glycosides and 6% terpenes or a placebo (capsules filled with ground Alfalfa leaves) at breakfast time. The chemical contents were also verified by HPLC analyses. The subjects returned to the GCRC for a second OGTT after the 3rd month. Subject compliance was greater than 96 during these studies, estimated by the returned capsules after every month. The subjects collected 24-hour urine specimens prior to the first and second OGTT visits. At each monthly follow-up, the subjects donated blood for clinical laboratory tests, completed questionnaires to document any changes in their health, symptoms, and any significant changes in their diet. In some studies, the euglycemic insulin clamp technique was performed to measure whole body insulin resistance.

Effect of *Ginkgo biloba* extract on metabolic syndrome

Initially, the experimental design was an open label in which the subjects ingested 120 mg daily, as a single dose, for three months. Healthy non-diabetic subjects exhibited reduced platelet reactivity as seen in platelet aggregation studies using platelet rich plasma in response to predominantly collagen and with no significant effect to PAF-induced aggregation [50]. The inhibition of platelet reactivity by *Ginkgo biloba* was confirmed by corresponding decrease of plasma TXB_2 in the platelet rich plasma and in urinary excretion of 11-dehydro-TXB_2, a metabolite of plasma TXB_2. As expected, the decrease in T2DM subjects was less than in healthy controls because of the increased platelet hyperactivity during the diabetic state. Pretreatment of harvested platelets with the *Ginkgo biloba* extract before exposure to the agonists again unexpectedly inhibited predominantly the aggregation promoted by collagen, rather than PAF. It was concluded that this is probably how it worked *in vivo* where, in a traumatized vascular endothelium, reduced activity with exposed collagen fibers in the subendothelial space, *Ginkgo biloba* could limit the risk and prevent the formation of thrombi. This could explain how *Ginkgo biloba* extract ingestion could increase blood flow. However, perhaps PAF was less effective because of its rapid hydrolysis by plasma acetylhydrolase. In a follow-up randomized double blind placebo control crossover study we confirmed that ingestion of *Ginkgo biloba* extract led to inhibition of COX-1 isoenzyme probably due to the flavonoid content which is known to contribute to its antioxidant and free radical scavenger properties [50]. In fact, ingestion of onion soup very rich in quercetin has also been shown to inhibit collagen-induced platelet aggregation, supporting our observation [51]. In other *in vitro* studies, washed platelets harvested from both diabetic and non-diabetic subjects were supplemented with exogenous arachidonic acid before and after pre-incubation with exogenous *Ginkgo biloba* extract. Ginkgo significantly inhibited not only the oxygenation of arachidonic acid to TXA_2 but also the production of thiobarbituric acid reacting (TBAR) substances, a marker of free radical/oxidative stress, by inhibiting COX-1 mediated malondialdehyde (MDA) production [52-54]. Thus, in both diabetic and healthy subjects, the ingestion of *Ginkgo biloba* extract reduced these indices. It is entirely possible that ingestion of *Ginkgo biloba* has effect both on the amount of arachidonic acid released by the plasma membrane as well as the COX-1 enzyme activity within the platelet.

Effect of *Ginkgo biloba* extract on pancreatic beta cell function

The standard oral glucose tolerance test (OGTT, with 75g glucose) was a very effective tool not only to confirm the absence or the presence of diabetes in the research subjects but also to assess the response of the pancreatic β-cell to the glucose challenge. Insulin, the hallmark of insulin resistance, is synthesized in pancreatic β-cells as part of a larger proinsulin molecule consisting of an A and B chains linked by a 31-amino acid connecting peptide (C-peptide) and two pairs of dibasic amino acids. In response to the glucose load, C-peptide is cleaved from the proinsulin molecule but remains in the secretory granules and is co-secreted with insulin. Thus, parallel and equimolar increases in both insulin and C-peptide are usually expected [55]. Interestingly, it was discovered that ingestion of *Ginkgo biloba extract* in healthy non-diabetic controls, enhanced pancreatic β-cell insulin production in response to the glucose challenge. Even more interesting was the fact that insulin and C-peptide levels were found not to be similarly increased [56]. Decreased plasma glucose was not observed in all cases and so those results indicated that *Ginkgo biloba* might have actually enhanced hepatic insulin extraction from the blood, compared to the slow renal elimination of C-peptide. In T2DM subjects, it was found that the ingestion of *Ginkgo biloba* extract produced a variable pancreatic β-cell response depending on the individual's insulin synthesizing capacity. In T2DM subjects that were hyperinsulinemic and to a lesser extent those with pancreatic exhaustion, both the early and late phases of insulin production in response to the glucose load at the start of the study were observed. In the diet and medication-controlled hyperinsulinemic T2DM patients, *Ginkgo biloba extract* produced no apparent significant change in pancreatic β-cell insulin secretory response or any significant changes in plasma glucose levels. Increased pancreatic β-cell function was however confirmed by a significant increase in plasma C-peptide [57]. In T2DM patients with pancreatic exhaustion on the other hand, ingestion of *Ginkgo biloba* extract caused a significant increase of pancreatic β-cell function reflected in significant increases in both plasma insulin and C-peptide and decreased plasma glucose.

Because hyperinsulinemia is a hallmark of insulin resistance, studies were undertaken to verify that ingestion of *Ginkgo biloba* extract did not increase insulin resistance. Using a robust randomized, double-blind, placebo-controlled crossover study and after ingesting either *Ginkgo biloba* extract (120 mg/day as a single dose) or placebo during each 3-month arm, a 2-step euglycemic insulin clamp technique [58] was performed in healthy non-diabetic, subjects with impaired glucose tolerance and those with overt T2DM subjects. Neither the low insulin infusion rate (10 $mU/m^2/min$) nor the high insulin infusion rate (40 $mU/m^2/min$) revealed any significant changes in glucose metabolic rates in any of the three groups as a result of the ingestion of the *Ginkgo biloba* extract. Thus, ingestion of *Ginkgo biloba* extract does not appear to increase insulin resistance [59].

Conclusion

The suspicion that arachidonic acid and platelet-activating factor together might be central to the development of diabetes and the complications of the metabolic syndrome could not be examined where it not for the Dietary Supplement and Health Education Act of 1994. The use of synthetic compounds like indomethacin (arachidonic acid inhibitor) or platelet-activating factor inhibitors, such as ginkgolide B or CV 3988 would have made these studies almost impossible to perform because of safety concerns. Taking advantage of *Ginkgo biloba* extract as a dietary supplement we have been able to make several important observations, including (a) inhibition of collagen-mediated platelet aggregation, (b) inhibition of platelet thromboxane synthesis, probably due to decrease of the arachidonic acid pool and or the inhibition of COX-1 enzyme activity, concomitantly with decreased oxidative stress, (c) *Ginkgo biloba* extract did not produce insulin resistance in the non-diabetic or pre-diabetic subjects or exacerbate the disease in the T2DM subjects, and perhaps importantly (d) for the first time, we were able to demonstrate the ability of *Ginkgo biloba* to stimulate pancreatic β-cell insulin production insulin production. In the case of T2DM subjects with pancreatic exhaustion, we cannot determine at this time whether *Ginkgo biloba*-stimulated insulin production is due to resuscitation of 'exhausted' pancreatic islets or merely an increased activity of the few remaining functional islets. It is noteworthy that we studied only 120 mg doses but they are several studies with higher doses. The recent debates on whether Alzheimer's disease is "Type 3 diabetes" is rather interesting given that diabetes and Alzheimer's disease share several common features, including insulin action and insulin resistance [59]. Perhaps the perceived *Ginkgo biloba* extract enhancement of memory by the general public may actually be related to enhanced insulin production and brain insulin signaling.

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