GCN2 and FGF21 are Likely Mediators of the Protection from Cancer, Autoimmunity, Obesity, and Diabetes Afforded by Vegan Diets

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Abstract

Third World quasi-vegan cultures have been characterized by low risks for "Western" cancers, autoimmune disorders, obesity, and diabetes. The relatively low essential amino acid contents of many vegan diets may play a role in this regard. It is proposed that such diets modestly activate the kinase GCN2 – a physiological detector of essential amino acid paucity – within the liver, resulting in up-regulated production of fibroblast growth factor 21 (FGF21). FGF21, by opposing the stimulatory effect of growth hormone on hepatic IGF-I production, may be responsible for the down-regulation of plasma IGF-I observed in vegans consuming diets of modest protein content. Decreased IGF-I bioactivity throughout life can be expected to have a favorable impact on cancer risk, as observed in rodents that are calorie restricted or genetically defective in IGF-I activity. Increased FGF21 in vegans might also contribute to their characteristic leanness and low LDL cholesterol by promoting hepatic lipid oxidation while inhibiting lipogenesis. Direct trophic effects of FGF21 on pancreatic beta-cells may help to explain the low risk for diabetes observed in vegans, and the utility of vegan diets in diabetes management. And up-regulation of GCN2 in immune cells, by boosting T regulatory activity, might play some role in the reduced risk for autoimmunity reported in some quasi-vegan cultures. The fact that bone density tends to be no greater in vegans than omnivores, despite consumption of a more "alkaline" diet, might be partially attributable to the fact that FGF21 opposes osteoblastogenesis and decreases IGF-I. If these speculations have merit, it should be possible to demonstrate that adoption of a vegan diet of modest protein content increases plasma FGF21 levels.

GCN2 – A Detector of Essential Amino Acid Paucity

GCN2 is a serine/threonine kinase which functions to detect a paucity of one or more essential amino acids, and to send a signal that enables an appropriate adaptive response.[1] Transfer RNAs (tRNAs) are usually in a "charged" configuration, covalently bound to their characteristic amino acids; however, when a specific amino acid is relatively deficient, the level of the uncharged tRNA specific to that amino acid increases. Uncharged tRNAs have the capacity to bind to GCN2, thereby activating its kinase activity.[2, 3] The activated GCN2 phosphorylates the eukaryotic initiation factor 2alpha (eIF2a) in such a way as to slow the translation of most mRNAs; however, this phosphorylation up-regulates the translation of certain select mRNAs coding for proteins that aid adaptation to essential amino acid deficiency. Most notably, translation of the transcription factor ATF4 increases, leading to increased transcription of a number of genes which are adaptive in this circumstance.[3, 4]

One of the genes whose transcription is boosted by ATF4 codes for the protein fibroblast growth factor 21 (FGF21); two conserved ATF4 binding sites are found in the promoter region of its gene.[5] Produced primarily in the liver, but also in adipocytes and skeletal muscle, FGF21 acts on the liver to suppress lipogenesis (by inhibiting maturation of SREBP-1) while activating hepatic fatty acid oxidation.[6-9] In mice fed a high-fat diet that typically induces obesity, concurrent administration of FGF21 exerts a

thermogenic effect and opposes weight gain, obesity, hepatic steatosis, and insulin resistance.[9] In part, these benefits appear to be mediated by increased production of adiponectin by adipocytes; adiponectin knockout mice are far less responsive to FGF21's impact on steatosis and insulin resistance.[10, 11]

Intriguingly, transgenic mice overexpressing FGF21 experience a significant increase in mean and maximal lifespan of around 30%.[12] This latter effect appears to be traceable, at least in part, to the fact that FGF21 inhibits the stimulatory impact of growth hormone on hepatic expression of IGF-I by blocking activation of the STAT5 transcription factor.[13, 14] (This effect appears to be part of a negative feedback loop, as GH increases transcription of FGF21 in the liver by activating STAT5.[15]) FGF21-mediated suppression of STAT5 activity could also be expected to increase hepatic expression of the IGF-I antagonist IGFBP-1.[16] It is well known that genetically altered mice in whom IGF-I production or activity is down-regulated enjoy an increase in maximal lifespan; the favorable impact of long-term calorie restriction in this regard may also reflect decreased IGF-I activity.[17, 18] GH receptor knock-out mice have much reduced plasma levels of IGF-I, and are characterized by reduced incidence and delayed occurrence of cancers.[19] Analogously, humans who are homozygous for a dysfunctional GH receptor – known as "Laron dwarves", as they have small stature – appear to be virtually immune to cancer.[20, 21] These observations are consistent with the principal that IGF-I, through its pro-proliferative and anti-apoptotic effects on many tissues, acts as a "universal cancer promoter".[22, 23]

Low Cancer Risk in Vegans – A Role for GCN2 and FGF21?

Quasi-vegan societies have been characterized by a marked reduction of the age-adjusted incidence of, and mortality from, many cancers common in Western society – so-called "Western" cancers such as those of the breast, prostate, colon, pancreas, ovary, and uterine endometrium.[24, 25] This phenomenon is however becoming less apparent as Westernized diets and lifestyles are adopted throughout the world. Figure 1 depicts some data pertinent in this regard, derived from a report by Wynder and colleagues.[26] It displays the age-adjusted death rates from the most common non-skin cancers in the U.S. in 1955, and contrasts them with the corresponding rates in Japan at that time. Post-war Japan was a relatively poor society, and a survey in the early 1950s estimated that only 6% of daily calories consumed were of animal origin, whereas 78% of calories came from grains.[27] Whereas the differences in lung cancer mortality are likely largely explicable by differences in tobacco use, the fact that *age-adjusted* mortality from cancers of the colon, prostate, breast, and ovary were 5-10-fold lower in Japan at the time is striking – yet quite consistent with other reports evaluating cancer rates in quasi-vegan societies. And even within the context of relatively low animal product consumption, a more vegan diet tends to associated with reduced cancer risk, as Colin Campbell's analysis of ecologic data from the China Study indicates.[28]

It is probably not coincidental that vegan diets of modest protein content are associated with a significant reduction of plasma IGF-I levels.[29-32] Surprisingly, moderate prolonged calorie restriction in humans does not appear to decrease IGF-I levels – in contrast to the down-regulatory impact of calorie restriction on IGF-I levels in rodents – but protein restriction does have this effect.[32] The molecular biology responsible for the down-regulation of IGF-I in vegans has heretofore remained obscure.

Vegan diets of modest protein content tend be relatively low, both on an absolute basis and a relative basis, in certain essential amino acids, most notably methionine and lysine.[33] Omnivore diets high in animal products are inherently high in protein, whereas vegan diets not unduly high in soy products, legumes, or foods compounded from isolated plant proteins tend to have a modest protein content.

Moreover, plant proteins tend to be lower in certain essential amino acids than most animal proteins are; in particular, legumes and nuts tend to be low in methionine, and grains characteristically are low in lysine.[33] Hence, most plant proteins are said to be of "low quality". It is reasonable to suspect that the relatively low content of certain essential amino acids in many vegan diets may play a mediating role in the lower IGF-I levels observed in vegans. In mice fed a methionine-restricted diet (one-seventh the methionine content of the control diet), plasma levels of FGF21 were found to be about 16-fold higher than in the controls, and plasma IGF-I was about 45% lower.[34] Although this study did not measure hepatic GCN2 activity, an increase of this activity reflecting low methionine availability seems likely to have been responsible, at least in part, for the marked up-regulation of FGF21 observed in methionine-restricted mice. I propose that, analogously, a vegan diet of moderate protein content induces a modest activation of hepatic GCN2, leading to an increase in FGF21 production that down-regulates hepatic production of IGF-I . A testable prediction of this model is that vegan diets capable of down-regulating plasma IGF-I will be associated with an up-regulation of FGF21.

Although American vegans appear to be at lower risk for cancer than are omnivores, the degree of protection they enjoy in this regard is not as intense as that that has been observed in quasi-vegan Third World cultures.[35] In part, this could reflect the fact that many American vegans only adopted such a lifestyle in midlife. Moreover, many American vegan diets are high in "faux meat" products rich in isolated plant proteins. A high intake of soy protein has been linked to increased IGF-I levels in vegans, possibly because soy protein is of intermediate quality.[31, 36] Also, many American vegans are not as lean as their Third World counterparts, owing to sedentary occupations and over-indulgence in foods high in added oils and sugars. (Potato chips, after all, are vegan!) Keeping protein intakes moderate, and maintaining a relatively lean physique by eating whole foods and getting appropriate exercise, are lifestyle strategies that should help vegans to minimize their cancer risk.

This hypothesis offers a straightforward explanation for the failure of dietary protein to emerge as a modulator of cancer risk in American case-control epidemiology. Only a minute fraction of the American public has been consuming a moderate-protein vegan diet for a large portion of their lives. Others most likely are eating a sufficient amount of high-quality protein to negate hepatic GCN2 activity. And once this activity has zeroed out, adding extra protein cannot reduce its activity further. That's why it is necessary to look to ecologic epidemiology, or to case-control studies from countries just emerging from a quasi-vegan traditional diet, to observe an impact of animal products or of protein *per se* on cancer risk. In this regard, a recent international ecologic analysis by Grant, examining age-adjusted incidence rates for 21 prominent types of cancer in 87 countries with high-quality data, concludes that energy derived from animal products and smoking are the most important modifiable (non-genetic) determinants of global cancer risk.[37] High-quality protein – in conjunction with other factors such as saturated fat, heme iron, bioavailable phosphate, and cooking-induced mutagens such as heterocyclic amines – seems likely to be a key mediator of the increased cancer risk associated with animal product consumption.

A vegan diet of modest protein content may also have potential for cancer management. Many cancers retain sensitivity to the growth-promoting and anti-apoptotic effects of IGF-I activity. Recently, Fontana and colleagues, working with human breast and prostate cancer xenografts in nude mice, showed that a reduction of dietary protein from 20% to 7% of calories was associated with a 56-70% reduction in cancer growth rates.[38] They also found that the prostate cancer grew 37% slower with a 20% plant protein diet than with 20% dairy protein diets. Serum obtained from patients who have followed the Pritikin program

of quasi-vegan diet and exercise for 11 days is less effective for promoting proliferation and inhibiting apoptosis in an androgen-sensitive prostate cancer cell line, than is their baseline serum, reflecting a lower content of IGF-I and a higher level of IGFBP-1.[39] Whereas it does not appear that down-regulating IGF-I levels is likely to achieve objective responses in human cancers – clinical attempts to regress cancers with antibodies targeting the IGF-I receptor have not been effective in this regard[40] – and cancers may be prone to evolve resistance to this strategy over time, nonetheless vegan diets may prove to be a useful adjuvant strategy for prolonging survival in some cancers. The venerable practice of using macrobiotic vegan diets in cancer management[41] – stigmatized as an unproven fad by many orthodox commentators[42] – may have been sustained by some genuinely favorable clinical experience.[43-45]

Whereas the impact of IGF-I on cancer risk and growth has been widely studied, the possible impact of FGF21 in this regard – independent of its impact on IGF-I, obesity, and insulin resistance – has been little studied. The fact that its key target tissues, aside from the liver – adipocytes, skeletal muscle, and pancreatic beta cells[46] – are unlikely to give rise to cancer, is reassuring in regard to the possibility of it have any direct cancer promoting activity. Moreover, since FGF21 is hepatoprotective in many circumstances, it would seem more likely to prevent hepatic cancer than encourage it.[47]

Does FGF21 Contribute to Leanness and Low LDL Cholesterol in Vegans?

If indeed moderate-protein vegan diets tend to up-regulate FGF21, this may have physiological implications beyond a reduction in IGF-I. In particular, vegans may be less prone to hepatic steatosis, and the tendency of FGF21 to promote hepatic fatty acid oxidation while opposing lipogenesis might play some role in the characteristic leanness of vegans.[48, 49] Whether FGF21 influences metabolic rate in humans might be doubted, as humans possess relatively little of the brown fat that functions as a thermogenic organ in rodents. However, a 20% increase in the respiratory rate of hepatocytes isolated from FGF21-treated mice has been reported, associated with accelerated oxidation of fatty acids and increased expression of carnitine palmitoyltransferase-1 and uncoupling protein-2;[50] the possibility that FGF21 might promote hepatic thermogenesis in humans is worthy of consideration. (Ironically, a high-protein diet also provides protection from hepatic steatosis in rodents!)[51]

An additional factor contributing to leanness and decreased cancer risk in vegans may be the relatively low ratio of saturated fat to unsaturated fat in most vegan diets.[52] This tends to promote relatively good muscle and hepatic insulin sensitivity; the consequent down-regulation of diurnal insulin secretion would be expected to oppose fat storage in adipocytes, and to up-regulate hepatic production of IGFBP-1, which binds to circulating IGF-I and decreases its effective bioactivity. Reduced adiposity, and lower plasma levels of insulin and free IGF-I, could be expected to decrease risk for a number of types of cancer. A reduced ratio of saturated fat to unsaturated fat is likewise characteristic of "Mediterranean" diets, and people who practice such diets tend to be leaner and less prone to many cancers than are people whose omnivore diets are relatively rich in saturated fats.[52]

The ability of FGF21 to inhibit triglyceride synthesis in hepatocytes could be expected to decrease hepatic secretion of apoB100, and hence lower the level of circulating LDL particles.[53, 54] Indeed, treatment of diabetic monkeys with FGF21 decreases their LDL cholesterol levels.[55] Conceivably, this phenomenon, in conjunction with a lower intake of saturated fat and a cholesterol-free diet (which tend to up-regulate hepatocyte expression of the LDL receptor), contributes to the characteristically low LDL cholesterol levels of vegans.[56-59]

A Role for FGF21 in Diabetes Prevention and Treatment?

In the prospective Adventist Health Study-2, vegans were found to be only 38% as likely (OR 0.381, CI 0.236-0.617) to develop diabetes after correction for appropriate covariates, including BMI.[60] However the vegans on average had a BMI 16% lower than the omnivores, and this may have been largely attributable to their diet; when BMI but not age was removed from the regression analysis, vegans were only 23% as likely to develop diabetes. Vegan diets have also been found to be useful in the management of diabetes.[61] The favorable impact of vegan diets on risk for obesity – possibly mediated in part by FGF21 - in conjunction with the relatively low ratio of saturated to unsaturated fat in most vegan diets, could be expected to have a favorable impact on insulin sensitivity and beta-cell lipotoxicity, and hence aid prevention and control of diabetes in vegans. However, FGF21 has been shown to act directly on beta-cells to prevent apoptosis;[46] apoptotic loss of beta-cells is a phenomenon which contributes to the onset and progression of diabetes. Moreover, short-term treatment of diabetic mice with FGF21 – too short to influence obesity – had a favorable effect on their pancreatic function and diabetic control.[46] Hence, the direct impact of FGF21 on the pancreatic islets may play some role in the favorable impact of vegan diets on risk for, and management of, diabetes.

Up-Regulation of GNC2 Activity May Protect Vegans from Autoimmunity

In the middle years of the twentieth century, the incidence of a number of autoimmune disorders tended to be quite rare in sub-Saharan black Africans, even though these disorders were not uncommon in American blacks.[62-64] Analogously, the incidence of certain autoimmune conditions was reported to be relatively low in Asian societies whose diets were quasi-vegan.[64] Vegan diets have also been shown to have some utility in the management of rheumatoid arthritis.[65-67] Could GCN2 activity be pertinent to this phenomenon?

There is evidence that the well-known impact of indolamine 2,3-dioxygnease (IDO) induction on immune tolerance and induction of T regulatory cells may be mediated at least in part by localized activation of GCN2 in immune cells. GCN2-knockout mice fail to undergo a remission phase when they are subjected to experimental autoimmune encephalomyelitis.[68] Effector cells (Th1, Th17) were higher, and T regulatory cells lower, in the CNS of these mice. Moreover, *in vitro* studies have shown that tryptophan depletion via IDO promotes T regulatory cell induction and foxp3+ expression in part via activation of GCN2.[69-71] Cobbold and colleagues have proposed that localized depletion of a wider range of essential amino acids via induction of catabolic enzymes within dendritic cells contributes to regulatory T cell induction, in part via reduction of mTORC1 activity in lymphocytes;[72] evidently increased activation of GCN2 might also play a role. It is reasonable to suspect that these phenomena would be up-regulated in vegans if baseline tissue levels of certain essential amino acids were relatively low. Hence, up-regulation of GCN2 activity might conceivably play a role in the reduced risk for autoimmunity enjoyed by certain quasi-vegan cultures.

A Cautionary Note on Bone Density

Transgenic mice with FGF21 overexpression, as well as mice whose high FGF21 levels are reflective of dietary methionine restriction, are characterized by low bone density.[34, 73] Although a decrease in IGF-I levels might contribute to this phenomenon, there is some evidence that a direct effect of FGF21 on marrow mesenchymal cells may inhibit osteoblastogenesis by up-regulating PPAR-gamma activity.[73]

Vegan diets of moderate protein content are characterized by a relatively low intake of the sulfur amino acids that are catabolized to yield sulfuric acid; hence, vegan diets, particularly those naturally high in potassium, tend to promote a systemic alkalinity that would be expected to slow the solubilization of bone mineral and aid maintenance of bone density.[74-76] Nonetheless, there is no consensus that vegans tend to have denser bones, and some studies even observe slightly lower bone density in vegans.[77-81] Conceivably, increased FGF21, either directly or via its impact on IGF-I,[82-85] exerts a countervailing negative effect on bone density in vegans - albeit lower calcium intake has also been suggested as a possible factor in the less than robust bone density of many vegans, and lighter weight might contribute as well. In any case, vegans should not presume that their "alkaline diet" provides them with special protection from osteoporosis, and should take whatever pharmaceutical, nutraceutical or lifestyle measures that are appropriate to protect their bone mass as they age.

Testing the Hypothesis

Although adoption of a protein-restricted vegan diet has been shown to decrease plasma IGF-I levels by about 30% in human volunteers, these studies did not measure the impact of such diets on FGF21 levels.[32] Documentation of such an effect would be one way to test the validity of the speculations offered here – albeit it would not prove that GNC2 mediated such an increase. Measuring GCN2 activity in blood leukocytes might be an additional way to assess whether practical levels of essential amino acid restriction in humans can up-regulate GCN2 and FGF21 activities.

An oddity of the emerging clinical literature on FGF21 is that FGF21 levels tend to be *increased* in people and in mice with obesity, metabolic syndrome, and hepatic steatosis, and can correlate positively with atherosclerosis.[86-91] This phenomenon may reflect, at least in part, the ability of increased free fatty acid exposure to induce FGF21 expression via PPARalpha activation; analogously, the free fatty acid elevation associated with prolonged fasting or acute exercise elevates FGF21.[92-95] This effect is evidently homeostatically appropriate, as FGF21 functions within the liver to promote catabolism of fatty acids. Intriguingly, oleate and linoleate induce FGF21 via PPARalpha, but palmitate does not;[96] this may be an additional reason why diets with a relatively high ratio of saturated to unsaturated fat tend to be more productive of obesity and diabetes. There is also evidence that both the liver and adipocytes of obese mice may be resistant to FGF21 signaling; in adipocytes, this may reflect the fact that TNFalpha decreases expression of β-klotho, a component of the FGF21 receptor.[97, 98] Yet, both in rodents and in diabetic monkeys, administration of FGF21 has a clearly favorable impact on adipose mass, insulin sensitivity, and steatosis.[9, 55, 99, 100] It is *not* proposed that vegans will have higher levels of FGF21; rather, it is proposed that, at least in the short term (before significant modification of body composition), adoption of a moderate-protein vegan diet will raise FGF21 levels.

If moderate-protein vegan diets do indeed boost FGF21 production in humans, a further line of inquiry would be to determine what adjuvant strategies might amplify this effect. Pharmaceutical PPARalpha agonists, histone deacetylase inhibitors, Sirt1 activators, and the drug metformin, may have some potential in this regard.[101-106] The clinical PPARalpha agonist bezafibrate has been reported to lower IGF-I levels in young men.[107] While it has been suggested that alternate-day fasting might elevate FGF21, and may be responsible for some of the metabolic benefits of this strategy in rodents,[108] two days of fasting did not increase FGF21 levels in humans – though a 78% increase was seen after 7 days of fasting.[93] Optimal strategies for increasing FGF21 may be especially fruitful for cancer prevention, as FGF21

suppresses hepatic production of IGF-I, while boosting that of IGFBP-1, promoting leanness and insulin sensitivity, and increasing adiponectin – all effects which can be expected to diminish risk for many cancers.[109]

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Figure 1

Age-Adjusted Cancer Mortality – U.S. vs. Japan - 1955

(Per 100,000 persons/year)

	Males		Females	
	U.S.	**Japan**	**U.S.**	**Japan**
Lung	92	16	11	7
Pancreas	20	7	11	4
Colon	28	6	31	6
Bladder	11	3	4	2
Breast	-	-	26	2
Ovary	-	-	25	3
Prostate	15	2	-	-
Lymphomas	14	5	8	2
Leukemias	13	3	9	2