Targeting Adipocytes in Metabolic Syndrome – Spirulina and Salsalate May Complement the Benefits of PPARgamma Agonists

Mark F. McCarty, Catalytic Longevity, markfmccarty@gmail.com

Abstract

Dysfunctional behavior of hypertrophied adipocytes is the fundamental driver of metabolic syndrome. Key features of this dysfunction include increased production of cytokines/adipokines that promote systemic inflammation and insulin resistance (such as IL-6, TNF-alpha, and resistin), decreased production of protective adiponectin, a dysregulation of fatty acid storage that encourages deposition of pathogenic ectopic fat in other tissues, and selective hypertrophy of visceral adipocytes. Adipocytes in metabolic syndrome are characterized by: oxidative stress stemming from NADPH oxidase activation; increased NF-kappaB activity; increased local generation of cortisol reflecting increased expression of 11-beta-hydroxysteroid dehydrogenase 1; diminished expression of the PPARgamma transcription factor; and a reduction of adipocyte insulin sensitivity. These factors interact in various ways to sustain each other and mediate the characteristic misbehavior of adipocytes in metabolic syndrome. Currently the only agents used for management of metabolic syndrome or diabetes that directly target adipocytes (aside from appropriate weight loss) are thiazolidinediones, potent agonists for PPARgamma; while pioglitazone notably ameliorates adipocyte function, side effects such as fluid retention, weight gain, and an adverse impact on bone formation render it less than ideal. Fortunately, there is reason to suspect that spirulina and salsalate may also favorably impact metabolic syndrome by modulating adipocyte behavior. Spirulina is a rich source of phycocyanobilin, which can function as a potent inhibitor of NAPDH oxidase, the key source of oxidative stress in adipocytes. Salsalate is a pharmaceutical delivery form of salicylate, which suppresses canonical NF-kappaB activation by directly inhibiting IKK-beta. These agents have the potential to complement the benefits of pioglitazone therapy in metabolic syndrome – or to substitute for it in patients intolerant to this drug – and hence merit further pre-clinical and clinical evaluation in this regard. Moreover, they may exert a range of other protective antioxidant/anti-inflammatory effects on target tissues in metabolic syndrome, including the liver, pancreatic beta cells, arterial endothelium, and foam cells. There is also intriguing recent evidence that the amino acids glycine and histidine may exert anti-inflammatory effects on adipocytes that might aid control of metabolic syndrome.

Adipocyte Dysfunction is at the Core of Metabolic Syndrome

Metabolic syndrome is rooted in a dysfunction of adipose tissue characterized by increased production and plasma levels of cytokines and adipokines that promote systemic inflammation, compromise insulin sensitivity of liver and skeletal muscle, inhibit fatty acid oxidation, and attract macrophages – notably IL-6, TNF-alpha, resistin, and MCP-1[1-3]- as well as decreased production of adiponectin, an adipokine which exerts anti-inflammatory and anti-atherosclerotic effects, promotes fat oxidation via activation of AMPK, and supports systemic insulin sensitivity.[4-8] The increased IL-6 acts on the liver to promote an acute phase response that contributes to the cardiovascular risk associated with metabolic syndrome;[9-13] increased hepatic production of serum amyloid A –rather than of C-reactive protein[14-16], a well-

characterized marker for cardiovascular risk – seems likely to mediate this risk.[17-21] Another key feature of metabolic syndrome is a dysregulation of fatty acid storage and release, mediated in large measure by adipocyte insulin resistance; as a result, serum free fatty acids remain inappropriately elevated postprandially owing to a relative failure of insulin to inhibit lipolysis, coupled with inefficient storage of meal-derived fat that further increases serum free fatty acids.[22, 23] Ironically, this may represent a homeostatic response that prevents animals from becoming counterproductively obese – at the cost of systemic insulin resistance and increased vascular inflammation. Exposure of muscle and other tissues to elevated free fatty acids, especially when insulin and glucose levels are increased and fatty acid oxidation is inhibited, leads to the production of "ectopic fat" metabolites such as ceramide and diacylglycerol which promote inflammation and oxidative stress while compromising insulin sensitivity.[24-30] In the liver, excessive delivery of free fatty acids is responsible for the increase in hepatic triglyceride synthesis and elevation of plasma triglycerides seen in metabolic syndrome. Ideally, adipose tissue should retain and store fat efficiently after meals, releasing fatty acids later in post-absorptive or fasting metabolism when fat is required by peripheral tissues as fuel; this appropriate regulation, which is disrupted in metabolic syndrome, minimizes the production of ectopic fat and prevents hypertriglyceridemia.

This sequence of events is typically triggered when adipocytes, particular those in visceral fat, become hypertrophied. Some of the key factors which appear to drive adipocyte dysfunction in metabolic syndrome include:

Increased oxidative stress in hypertrophied adipocytes, stemming from activation of NADPH oxidase coupled with a reduced expression of antioxidant enzymes.[31, 32] Activation of NADPH oxidase in hypertrophied adipocytes may reflect excessive glucose permeability, stemming from increased expression of GLUT1, that in turn activates PKC-delta, likely owing to increased production of diacylglycerol.[31, 33] PKC-delta in turn can activate NADPH oxidase in adipocytes, and the resulting production of oxidants further activates PKC-delta, promoting a vicious cycle that amplifies oxidative stress.[33] Oxidative stress works in a number of ways to promote the adipocyte dysfunction characteristic of metabolic syndrome, and may in fact trigger the syndrome via up-regulation of NF-kappaB activity.[34] Apocynin, a phytochemical inhibitor of NADPH oxidase complexes, has a marked ameliorative impact on adipocyte function and the systemic effects of metabolic syndrome in diabetes-prone KKAy mice and in rats given a fructose-rich diet; these result attest that NADPH oxidase overactivation in adipocytes plays a central role in metabolic syndrome.[32, 35]

Overactivation of adipocyte NF-kappaB in metabolic syndrome leads to increased transcription and synthesis of the pro-inflammatory cytokines/adipokines IL-6, TNF-alpha, resistin and MCP-1.[36, 37] TNF-alpha works in an autocrine fashion to further boost NF-kappaB activation – an effect which is potentiated by oxidative stress.[34] Furthermore, MCP-1 secretion promotes the migration of macrophages to adipose tissue, which in turn further contribute to a stew of inflammatory cytokines that impact adipocyte function adversely.[38, 39] NF-kappaB activation in adipoyctes may also be a key driver of 11-beta-hydroxysteroid dehydrogenase 1 (HSD1) expression, which boosts corticosteroid activity in adipocytes by promoting reduction of inactive cortisone to active cortisol.[40, 41]

Increased expression of HSD1 activity in adipocytes boosts autocrine cortisol production, which selectively promotes the hypertrophy of visceral adipocytes, possibly owing in part to an increase in the lipoprotein lipase activity of these cells.[42-47] In transgenic mice which experience marked reduction of

cortisol level owing to adipocyte-specific expression of HSD2 (which tends to convert cortisol to cortisone), adipocyte insulin sensitivity is improved, expression of resistin and leptin in diminished, and expression of adiponectin and PPARgamma is boosted;[48] this likely implies that the excess of adipocyte cortisol associated with metabolic syndrome has the opposite effect – compromising adipocyte insulin sensitivity, suppressing expression of PPARgamma, and promoting the pattern of cytokine/adipokine production that typifies metabolic syndrome. It is reasonable to suspect that oxidative stress, complementing the autocrine/paracrine activity of TNF-alpha, plays a role in driving increased adipocyte expression of HSD1 in metabolic syndrome, as oxidative stress up-regulates TNF-alpha-mediated activation of NF-kappaB.[34, 40, 49]

Decreased expression of PPARgamma in adipocytes is a feature of metabolic syndrome, and may reflect the joint impact of oxidative stress and of cortisol excess; how these factors compromise PPARgamma expression is still unclear.[32, 48] PPARgamma activity plays a crucial role in adipogenesis and in supporting the proper function of adipocytes; the multiple favorable effects of thiazolidinedione activators of PPARgamma on adipocytes in individuals with metabolic syndrome or diabetes – improved adipocyte insulin sensitivity, a suppression of HSD1 expression, and a more normal pattern of cytokine/adipokine production – bespeak the central role of PPARgamma in promoting physiologically appropriate adipocyte function.[50, 51] In particular, PPARgamma is the key transcription factor which mediates adiponectin expression.[52] PPARgamma also increases the expression of various enzymes and transporters required for fatty acid uptake and storage; this promotes insulin sensitivity peripherally by alleviating free fatty acid overexposure, but necessarily is associated with some increase in adipose mass.[53, 54] Increased expression of GLUT4 and of glucokinase in adipocytes is partially responsible for the favorable impact of PPARgamma agonists on adipocyte insulin sensitivity, and aids efficient triglyceride synthesis.

Impaired adipocyte insulin sensitivity in metabolic syndrome may reflect the joint influences of oxidative stress, cortisol excess, and PPARgamma down-regulation. Adipocyte oxidative stress may impair insulin sensitivity by up-regulating cytokine-mediated activation of JNK and IKKbeta, each of which can induce inhibitory phosphorylations of IRS-1, key mediator of the insulin signal.[55-58] Oxidative stress may also act less directly in this regard by boosting cortisol levels and suppressing PPARgamma activity.

Thiazolidinediones – Helpful but Flawed

Aside from appropriate weight loss that alleviates adipocyte hypertrophy, the only agents directly targeting adipocytes that are used for control of metabolic syndrome are the thiazolidinediones, of which pioglitazone is the safest. Nonetheless, these agents act on the kidney to promote water retention – a particular problem for patients at risk for congestive failure – and they also promote a modest amount of weight gain.[59, 60] Evidence has also emerged that they can have an unfavorable impact on bone density by opposing osteoblastogenesis.[61] Thiazolidinediones are therefore less than ideal for widespread use, especially in primary prevention. Fortunately, there is reason to suspect that two other agents – the phycocyanobilin (PhyCB) derived from spirulina, and salsalate, a dimer of the natural anti-inflammatory agent salicylate – may have potential for alleviating the adipocyte dysfunction associated with metabolic syndrome.

Bilirubin and Phycocyanobilin Target Oxidative Stress in Adipocytes

The bilirubin derived from induction of the antioxidant enzyme heme oxygenase-1 (HO-1) is now known to function physiologically as an inhibitor of certain isoforms of NADPH oxidase.[62-65] (Its isoform specificity still requires clarification.) This may explain why, in fat-fed mice, systemic induction of HO-1, or increased adipocyte-specific expression of HO-1 achieved by intracardial injection of a lentiviral vector (aP2-HO-1), is associated with a marked improvement of adipocyte function and improved systemic insulin sensitivity.[66-69] The inverse association of serum bilirubin with metabolic syndrome observed in many epidemiological studies, may imply that increased inducibility of HO-1 helps to prevent and control this syndrome.[70-76] Indeed, high expression alleles of the polymorphic HO-1 gene have been linked to reduced risk for type 2 diabetes in a Chinese population.[77] Also, serum bilirubin is reported to correlate inversely with visceral adiposity (though not overall body fat); arguably, this could reflect a suppressive impact of bilirubin on HSD1 induction in visceral adipocytes.[78]

Phycocyanobilin (PhyCB), a prominent chromophore in cyanobacteria such as spirulina, is a derivative and homolog of bilirubin's precursor biliverdin, and there is recent evidence that within cells it can mimic the NADPH oxidase-inhibitory activity of bilirubin (likely after intracellular conversion to the bilirubin homolog phycocyanorubin).[79, 80] This likely explains the profound and versatile anti-inflammatory activity of orally administered spirulina or phycocyanin (the spirulina protein to which PhyCB is covalently attached) in rodent studies.[79, 81] In KKAy diabetes-prone mice, orally administered phycocyanin has a favorable impact on systemic markers of metabolic syndrome such as insulin sensitivity, glucose tolerance, and plasma and hepatic lipid profile.[82] In fructose-fed rats, feeding spirulina concurrently improves blood glucose, serum lipid profile, and liver function markers.[83] Clinically, in Korean type 2 diabetics, ingestion of 8 g spirulina daily, while it did not notably influence glycemic control, was associated with a reduction in plasma triglycerides and an increase in plasma adiponectin.[84] Reductions of serum triglycerides have also been reported in non-diabetic subjects and in obese mice ingesting spirulina, likely reflecting an improvement in adipocyte insulin sensitivity.[85-87] Administered at 19 g daily, a markedly favorable impact of dietary spirulina on insulin sensitivity was noted in African HIV patients who had developed a Cushingoid-lipodystrophy syndrome associated with HAART therapy;[88] conceivably, this reflects, in part, a down-regulation of the elevated adiopocyte HSD1 expression characteristic of this syndrome.[89] It has been estimated that consumption of 15-30 g spirulina daily might be required to replicate clinically the anti-inflammatory effects of spirulina or phycocyanin documented in rodents.[79] The clinical impact of ample intakes of spirulina or phycocyanin on metabolic syndrome clearly should receive further evaluation; development of PhyCB-enriched spirulina extracts as a nutraceutical would evidently expedite such efforts, as most people dislike the flavor and odor of spirulina.

Anti-inflammatory Amino Acids – Glycine and Histidine

The amino acid glycine, in concentrations achievable with multi-gram oral doses, lessens NADPH oxidase activity in macrophages and certain other cells by hyperpolarizing the plasma membrane; it does so by activating glycine receptors that promote chloride influx.[90, 91] When 3T3-L1 cells are differentiated to adipocytes in vitro, concurrent exposure to glycine is associated with down-regulated expression of IL-6, resistin, and TNF-alpha, and up-regulated expression of adiponectin and PPARgamma – precisely the

pattern seen in diabetes-prone rodents treated with apocynin.[92] In sucrose-fed rats, glycine feeding decreases adipocyte size, plasma free fatty acids, and blood pressure.[93] Glycine, which has a pleasantly sweet flavor and is inexpensive, has been administered to human diabetics in a dose of 5 g 3 times daily; a marked reduction in glycated hemoglobin was noted, apparently reflecting glycine's ability to suppress protein glycation.[94, 95] The impact of high-dose glycine on metabolic syndrome merits further evaluation, in rodents and in humans; its effects on adipocytes might be similar to those of spirulina.

Recent evidence indicates that histidine may also exert anti-inflammatory effects on adipocytes. When cultured adipocytes were exposed to palmitic acid to induce insulin resistance and activate NF-kappaB, concurrent exposure to histidine dose-dependently inhibited induced expression of IL-6 and TNF-alpha, while aiding preservation of insulin sensitivity.[96] In overweight women treated with 2 g histidine twice daily for 12 weeks, or a matching placebo, histidine supplementation was associated with significant reductions in serum IL-6, TNF-alpha, and HOMA-IR, whereas adiponectin rose by over 30% from baseline.[96] A 2.8 kg loss of body fat was also observed in the histidine-treated group, which might have contributed to the amelioration of adipocyte function. Other recent studies shows that histidine dose-dependently blunts TNF-alpha-mediated activation of NF-kappaB in cultured endothelial cells and in a monocytic leukemia cell line.[97, 98] Analogously, histidine exposure blunts LPS-mediated NF-kappaB production and cytokine induction in macrophages, and orally administered histidine was protective in a murine colitis model.[99] And in Balb/cA diabetic mice, oral histidine suppressed elevations of serum IL-6 and TNF-alpha[100] – in nice concordance with the clinical trial cited above. The molecular basis of histidine's anti-inflammatory activity in these studies remains obscure, although antioxidant effects of histidine or its derivative carnosine have been reported. [100-103]

Salicylate Suppresses NF-kappaB Activation in Adipocytes

Salsalate, a pharmaceutical delivery form for the anti-inflammatory phytochemical salicylate, likewise has the potential to ameliorate metabolic syndrome via direct effects on adipocytes. This reflects its ability, in clinically feasible concentrations, to diminish the activity of IKK-beta, which mediates activation of NF-kappaB via the canonical pathway in cytokine-exposed adipocytes.[104, 105] When cultured adipocytes are exposed to inflammatory factors secreted by LPS-stimulated macrophages, adipocyte expression and secretion of TNF-alpha, IL-6 and resistin all increase, whereas expression of PPARgamma and of adiponectin is suppressed; concurrent exposure to clinically-relevant concentrations of salicylate reverses these effects.[37] In patients with impaired glucose tolerance, administration of 3-4 g salsalate daily was associated with a 53% increase in plasma adiponectin and a 25% reduction in plasma triglycerides; a biopsy study revealed a small but significant reduction of NF-kappaB activity in subcutaneous adipocytes.[106] However, no improvement of systemic insulin sensitivity, assessed by euglycemic insulin clamp, was noted in this study. Salsalate treatment was found to decrease adipocyte HSD1 expression in men and in fat-fed mice[107] – not surprising in light of the role of NF-kappaB in induction of this enzyme in adipocytes.[40] Salsalate improves glycemic control in type 2 diabetics, but this effect may be at least partially attributable to an unexplained reduction in hepatic insulin clearance.[106, 108-110] The clinical effects of salsalate would likely be more impressive if reversible ototoxicity – tinnitus, mild hearing impairment – did not place an upper limit on the feasible clinical dose, limiting the extent to which NF-kappaB activity can be suppressed in vivo.[111, 112] (Most people tolerate 3 g daily, but tinnitus becomes a more prominent complication at 4.5 g a day.[106]) Concurrent administration of optimal doses of

spirulina would seem likely to potentiate salsalate-mediated inhibition of NF-kappaB by blunting the impact of oxidative stress.

It would be of particular interest to determine how joint administration of spirulina and salsalate might complement the utility of pioglitazone in management of metabolic syndrome or diabetes – or how well this combo might substitute for piogliazone in patients intolerant to this agent.

Spirulina and Salsalate Have Versatile Potential in Metabolic Syndrome

While spirulina and salsalate can target adipocyte metabolism, they may also exert anti-oxidant/anti-inflammatory effects on other tissues or cells whose function is adversely impacted in metabolic syndrome – such as the liver, vascular endothelium, foam cells, and pancreatic beta cells; this reflects a key role for NADPH oxidase and NF-kappaB in mediation of the complications of metabolic syndrome and diabetes.[113-122] Hence, while these agents may have the potential to "get to the root of the matter" by direct effects on adipocytes, they may also lessen the downstream adverse effects of metabolic syndrome on systemic physiology. Notably, salicylate as well as spirulina/phycocyanin exert anti-atherosclerotic effects in rodent studies, oral administration of PhyCB or phycocyanin was recently reported to prevent glomerulosclerosis in diabetic mice, and pilot clinical experience, as well as rodent studies, suggest that dietary spirulina may be useful in non-alcoholic fatty liver disease, a frequent complication of metabolic syndrome.[80, 114, 123-128]

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