Perspectives in Diabetes

A Burning Question

Does an Adipokine-Induced Activation of the Immune System Mediate the Effect of Overnutrition on Type 2 Diabetes?

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There is growing support for the hypothesis that obesity is an inflammatory condition leading to chronic activation of the innate immune system, which ultimately causes progressive impairment of glucose tolerance. Experimental studies in animals and evidence from prospective and longitudinal studies in humans are consistent with an etiologic role of subclinical inflammation in the pathogenesis of type 2 diabetes, primarily as a mediator of obesity-induced insulin resistance. However, the exact chain of molecular events linking overnutrition, activation of the innate immune system, and impairment of insulin signaling in peripheral tissues remains incompletely understood. Notwithstanding this limitation, treating the underlying subclinical inflammation may constitute a novel approach to prevention and/or treatment of type 2 diabetes. *Diabetes* 54:917–927, 2005

Through the story of evolution, animals and humans have developed redundant mechanisms that promote the accumulation of fat tissue during periods of "feast," thus enabling survival during periods of "famine" (1). However, what once was an asset has become a liability in the current "obesigenic" environment of readily available high-energy foods and little need for physical activity. As a consequence, obesity has reached epidemic proportions in both industrialized and developing countries around the world, which is a major public health problem because obesity is associated with significant comorbidities and increased mortality.

Clinicians have long observed that fatter people are more likely to develop type 2 diabetes, and overwhelming scientific evidence has proven this clinical impression to be accurate. The association of obesity with type 2 diabetes has been observed in comparisons of different populations and within populations. Prospective studies of prediabetic subjects have conclusively shown that obesity and its duration are major risk factors for type 2 diabetes. Despite the remarkable consistency of the association between the two diseases, obesity is neither sufficient nor necessary for the development of type 2 diabetes. For example, many U.S. whites are overweight or obese, but <10% of this population has type 2 diabetes.

How does obesity cause type 2 diabetes and why in only certain people? Experimental weight gain results in hyperinsulinemia and insulin resistance in animals and humans. It is clear how type 2 diabetes develops in the absence of insulin secretion, but how does insulin resistance gradually result in the disease?

The concepts of glucotoxicity, lipotoxicity, and cellular nutrient overload to explain the pathogenesis of type 2 diabetes in obese individuals have been advanced previously, but these theories have evidently failed to provide a universally accepted and pathophysiologically conclusive explanation that would link excessive adiposity to insulin resistance and insulin secretory dysfunction. Thus, new theories continue to emerge.

In this review, we will present the growing body of evidence indicating that obesity may be an inflammatory condition leading to chronic activation of the innate immune system, which ultimately causes progressive impairment of glucose tolerance and eventually type 2 diabetes.

ORIGIN OF THE HYPOTHESIS

The theory that inflammation may be involved in the pathogenesis of type 2 diabetes is not new. The first indication of this pathophysiological connection can be traced to Ebstein (2) who, >100 years ago, reported in the German scientific literature that high doses of salycilate improved glycosuria in diabetic patients. This idea was then forgotten until a group of epidemiologists in the mid-1990s discussed the possibility that diabetes and atherosclerosis, an inflammatory condition in its own right, have common antecedents (the "common soil hy-

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AP, activating protein; FFA, free fatty acid; IKK, IκB kinase; IL, interleukin; IRS, insulin receptor substrate; JNK, Jun NH_2-terminal kinase; NF-κB, nuclear factor-κB; PI, phosphatidylinositol; TNF-α, tumor necrosis factor-α; TZD, thiazolidinedione.

pothesis" [3]). But it was the 1998 publication "Is Type II Diabetes Mellitus a Disease of the Innate Immune System?" by Pickup and Crook (4) that finally laid out a more specific pathophysiological hypothesis. Based on the observation that the dyslipidemia common to people with type 2 diabetes (high triglycerides and low HDL cholesterol) is also a feature of experimental and naturally occurring acute-phase reactions, Pickup and Crook proposed that in individuals with an innately hypersensitive acute-phase response, long-term lifestyle and environmental stressors, such as nutrition, produce disease (type 2 diabetes) instead of repair.

Pickup and Crook explained that the innate immune system, a rapid first-line defense system based on nonlymphoid tissue, is primarily responsible for the acute-phase response, a self-limiting process induced by a variety of stressors (infection, tissue injury, and malignancy) causing a number of cells (macrophages, adipocytes, and endothelial cells) to secrete cytokines (interleukin [IL]-1, IL-6, and tumor necrosis factor-α [TNF-α]), which act on the liver to synthesize acute-phase proteins (fibrinogen, C-reactive protein, serum amyloid A, and others). Due to its self-limiting nature, the acute-phase response is aimed at restoring the homeostasis disturbed by an acute stressor. However, in response to chronic stressors, the system may become allostatic, i.e., the sustained effort to acutely battle challenges may ultimately result in an overload of the system resources. Eventually when the allostatic load exceeds these resources, the system breaks down.

While brilliant, Pickup and Crook's theory had a few shortcomings. It was based primarily on cross-sectional observations, and, although it predicted that the most likely chronic stressors are nutritional ones, it did not explain how this could result in increased secretion of cytokines by multiple cell types and gave no molecular explanation as to how these cytokines could inhibit insulin action in peripheral tissues and/or glucose-stimulated insulin secretion in the pancreas.

CHRONIC ACTIVATION OF THE IMMUNE SYSTEM AND TYPE 2 DIABETES: CAUSE OR CONSEQUENCE?

A number of studies (5,6) have reported increased acute-phase proteins and other nonspecific markers of inflammation in type 2 diabetes. This is not particularly surprising, since inflammatory processes in affected tissues accompany some of the chronic complications of type 2 diabetes. However, studies in nondiabetic individuals have challenged this interpretation.

Healthy people who go on to develop diabetes, when compared with those who remain nondiabetic, are more obese (particularly centrally), insulin resistant, and have abnormal insulin secretory function (7). Interestingly, obesity and insulin resistance seem to be positively associated with elevated markers of inflammation in most studies, whereas no convincing evidence of a relationship between insulin secretory dysfunction and inflammation has been reported. Obesity was found to be associated with nonspecific measures of activation of the immune system, such us total γ-globulin concentration (8), body temperature (9), white blood cell count (10), and C-reactive protein (11). Associations between fibrinogen and clinical features of the metabolic syndrome (12), as well as associations between oral temperature or white blood cell count and insulin sensitivity (10,13,14), have also been reported. Because in most (15–22) if not all (23,24) cases the association between inflammatory markers and insulin resistance was found to be independent of adiposity, it has been suggested that inflammation is a possible pathophysiological link between obesity and insulin resistance. However, whether or not inflammation is a pre-diabetic abnormality cannot be determined from cross-sectional studies alone. Such conclusions are better drawn from prospective and longitudinal studies.

Many prospective studies (8,20,25–36), in diverse human populations, have identified proinflammatory cytokines, acute-phase proteins, and several indirect markers of inflammation as predictors of type 2 diabetes. This predictive effect of inflammation on the risk of type 2 diabetes does not seem to depend on subclinical cardiovascular disease (25,30,32,35), undiagnosed diabetes at baseline (8,20,29–31,33,36), or, surprisingly, initial degree of insulin resistance (20,25,27,29–32,35,36). Although this association is substantially lessened by obesity, in most of the studies at least one of the inflammatory markers identifies people at risk of diabetes independent of their degree of adiposity or upper body fat distribution (Table 1). Very few longitudinal studies have investigated whether inflammation may cause diabetes by reducing insulin sensitivity and/or insulin secretory function. We addressed this issue in a study (20) of 81 Pima Indians with normal glucose tolerance at baseline, after adjustment for several covariates including concomitant changes in adiposity, and found that white blood cell count was associated with a decline in insulin sensitivity but not insulin secretory function.

Thus, while an inconsistent use of inflammatory markers to biochemically define chronic activation of the immune system makes it very difficult to summarize this body of literature, it seems that the evidence from prospective and longitudinal studies is consistent with an etiologic role of inflammation in the pathogenesis of type 2 diabetes, primarily as a mediator of obesity-induced insulin resistance (Fig. 1*A*). Pickup (37) reached a similar conclusion in a recently published reappraisal of the original 1998 theory.

OVERNUTRITION AND CHRONIC ACTIVATION OF THE INNATE IMMUNE SYSTEM: THE ROLE OF ADIPOKINES

The traditional view of adipose tissue as a passive energy storage depot was challenged when it was identified as a major site for the metabolism of steroid hormones (38), and it is no longer valid after the discovery that adipose tissue secretes a number of bioactive proteins. These proteins, known as adipokines, have local autocrine/paracrine effects as well as systemic hormonal effects and span a vast array of chemical structures and functional classes (Fig. 2), as recently reviewed by Kershaw and Flier (39).

Why is obesity associated with inflammation? Pickup and Crook's (4) prediction of nutritional factors as a chronic activator of the innate immune response makes sense if one extends the concept to include overnutrition and the resulting increase in adiposity. Thus, the simplest explanation for why obesity is associated with inflammation is that the hyperplastic/hypertrophic expansion of the adipocyte mass results in altered circulating levels of proinflam-

matory cytokines. Other hypotheses include elevated local production of TNF-α, which is both a local adipostatic signal (inhibitor of lipoprotein lipase) and a trigger of the inflammatory response. Adipose tissue expansion, like tumors, is angiogenesis dependent (40). It has been suggested that adipocytes may become hypoxic during a rapid expansion of adipose tissue and start secreting inflammatory cytokines, which serve to increase blood flow, and some of them (such us leptin and vascular endothelial growth factor) may directly stimulate angiogenic factors (41). Adipose tissue from obese humans has been shown to have increased 11β-hydroxysteroid dehydrogenase type 1 activity (42). In rodents, selective overexpression of 11β-hydroxysteroid dehydrogenase type 1 in adipose tissue is accompanied by proinflammatory changes in the adipokine expression profile (leptin, resistin, adiponectin, and TNF-α) (43). Finally, adipose tissue is now recognized as a complex organ containing adipocytes as well as connective tissue matrix, nerve tissue, stromovascular cells, and immune cells. Lately, the presence of immune cells has received increased attention owing to significant functional and molecular overlap between adipocytes and macrophages, as in the recent discovery that adipocyte precursors can be transformed into macrophage-like cells in response to the appropriate stimuli (44) and that adipose tissue, but not liver or muscle, in obese people is characterized by macrophage infiltration (45,46). Increased levels of monocyte chemotactic protein-1 secreted by adipocyte or preadipocytes in response to TNF-α could be one of the chemokines implicated in this recruitment of macrophages, which would perpetuate the inflammatory response.

Interestingly, adipose tissue secretes proteins that have both stimulatory and inhibitory effects on the inflammatory process. Among the proinflammatory adipokines, TNF-α and IL-6 have been studied most extensively. The effects of TNF-α on glucose metabolism may be mediated in an autocrine/paracrine manner by regulating secretion of other adipokines or by promoting lipolysis and raising serum free fatty acid (FFA) levels. TNF-α is overexpressed in different models of murine (47) and in human obesity (48,49), whereas weight reduction decreases its expression and/or plasma concentration (48–50). In humans, a single bolus intravenous injection of recombinant human TNF-α increased plasma glucose concentrations and plasma FFAs (51); TNF-α neutralization affects direct measures of whole-body insulin sensitivity in rats (47) but not in humans (52).

While TNF-α in human plasma has been found at very low concentrations, adipose tissue accounts for 30% of the circulating IL-6, suggesting an endocrine role for this adipokine (53). In the liver, IL-6 is the primary stimulator for the production of most of the acute-phase proteins (54). IL-6 in vitro reduces insulin-stimulated insulin receptor substrate (IRS)-1 tyrosine phosphorylation, as well as IRS-1–associated phosphatidylinositol (PI) 3-kinase activity (55), and in mice, IL-6 treatment causes insulin resistance in skeletal muscle and in liver most likely due to defects in IRS-1 (and IRS-2, respectively)-associated PI 3-kinase activity (56). In humans, IL-6 is related to insulin resistance, independent of obesity (57,58). In the Atherosclerosis Risk in Communities study (26) and Nurses Health study (30), IL-6 at baseline was independently associated with future risk of diabetes (Table 1). Similar results were reported in the European Prospective Investigation into Cancer and Nutrition–Potsdam study (33), in which participants with elevated IL-6 and IL-1β had a threefold increase in risk for developing diabetes when compared with the reference group.

Other proinflammatory adipokines have been studied. Leptin, in vitro, has been found to have proinflammatory properties (59) and may promote monocyte diapedesis and the accumulation of macrophages in adipose tissue (60). However, because leptin improved insulin sensitivity in rodents (61) and in humans (62) with lipodystrophy, this makes it an unlikely contributor to the inflammatory response associated with obesity. Resistin was originally reported as an adipose tissue–specific hormone that provided a link between obesity and diabetes. Resistin is part of a new class of cysteine-rich secreted proteins that were found, by one of the groups who discovered it, to be induced during lung inflammation (found in inflammatory zone 1 [FIZZ1]). In vitro studies have shown that resistin mRNA expression is increased by proinflammatory cytokines in human mononuclear cells (63) and that resistin has a direct proinflammatory effect, probably mediated through nuclear factor-κB (NF-kB) signaling pathway on vascular endothelial human cells (64). In rodents, resistin affects glucose tolerance and is related with whole-body and hepatic insulin resistance (65,66). Differences in resistin gene expression have been observed between human and mice tissues. While in mice adipocytes are the mayor source of circulating resistin, in humans secretion or expression is predominantly found in mononuclear cells, and the release of resistin by explants of adipose tissue in primary culture is largely derived from the nonfat cells present in the explants (67). Resistin is detectable in human serum, and its circulating levels were found to be elevated in proportion to the degree of adiposity (68,69). Although these data might suggest a contribution of resistin to inflammation and insulin resistance, the role of resistin in obesity has not been proven (68,69). Finally, other proinflammatory adipokines, such as complement C3 and macrophage inhibitor factor, were inversely and independently associated with insulin sensitivity (70,71).

Adiponectin is the anti-inflammatory adipokine that has been studied most extensively. It is produced exclusively by white adipocytes but is paradoxically lower in obese versus lean individuals (72). Adiponectin has been related to insulin resistance and diabetes not only because of its AMP-activated protein kinase effects on FFA metabolism and glucose uptake but also because of its anti-inflammatory properties. Inhibition of phagocyte activity and TNF-α production by macrophages and inhibition of the TNF-α–induced expression of adhesion molecules (through NF-κB signaling pathways) are some of the known mechanisms by which adiponectin mediate its anti-inflammatory effects (73). Many studies have now suggested that in humans adiponectin is more closely related to insulin resistance than to obesity (73). Prospective and longitudinal studies (74–78) have found a correlation between low adiponectin levels and a higher risk of diabetes, independent of many confounders including obesity and other inflammatory markers (75,79). Krakoff et al. (79) hypothesized that in

TABLE 1
Effect of inflammation on the risk of type 2 diabetes: prospective studies

Authors (ref.) (*n*/CTD/FU)	Inflammatory markers*	Confounders accounted for in final model	Risk of developing type 2 diabetes (95% CI)	Comments (*n*)
Schmidt et al. (32) (12,330/1,335/7)	White blood cell count (albumin, fibrinogen)	Age, sex, center, smoking, physical activity, familial history of diabetes, fasting glucose, waist-to-hip ratio, BMI	OR (4Q vs. 1Q) = 1.5 (1.3–1.8)	White blood cell count showed similar association if cartoid internal median thickness included in the model
Schmidt et al. (32) (610/33/5)	Orosomucoid, sialic acid (haptoglobin, α1 antrypsin)	Age, sex, center, smoking, ethnicity, atherosclerosis, familial history of diabetes, fasting glucose, waist-to-hip ratio, BMI	Orosomucoi: OR (4Q vs. 1Q) = 7.1 (2.1–23.7); sialic acid: OR (4Q vs. 1Q) = 2.8 (1–8.1)	Stronger associations within the first 3 years of follow up
Barzilay et al. (25) (3,223/45/3–4)	C-reactive protein (white blood cell count, platelet count, albumin, fibrinogen, factor VIIIc)	Age, sex, subclinical cardiovascular disease, fasting glucose, fasting insulin, BMI	OR (4Q/1Q) = 1.8 (1.2–2.9)	Subjects with normal fasting glucose at baseline
Lindsay et al. (8) (1,694/568/17)	γ-Globulin	Sex, age, BMI, 2-h glucose	HR 1.14 (1.1–1.2)	Normal glucose tolerance subjects at baseline; results in impaired glucose tolerance = NS
Pradhan et al. (31)	C-reactive protein (IL-6)	(Matched by age and fasting status.) Physical activity, smoking, alcohol, hormone replacement therapy, BMI, fasting insulin	RR (4Q vs. 1Q) = 4.3 (1.1–17.1)	Nested case (126) control (255) study with fasting samples and HbA_{1c} <6.5%
Festa et al. (27) (1,047/144/5)	Plasminogen activator inhibitor-1 (C-reactive protein, fibrinogen)	Age, sex, center, ethnicity, smoking, physical activity, familial history of diabetes, fasting glucose, BMI, insulin sensitivity	OR (for 1 SD increase) = 1.32 (1.–1.7)	Stratified analysis (oral glucose tolerance test at baseline); results in impaired glucose tolerance = NS
Freeman et al. (28) (5,245/151/5)	C-reactive protein (white blood cell count)	Age, smoking, alcohol, triglycerides, total cholesterol, HDL cholesterol, white blood cell count, systolic blood pressure, BMI, fasting glucose, pravastastin treatment	OR (5Q vs. 1Q) = 2.46 (1.2–5.04)	Criteria for diabetes at follow up: two fasting glucose levels ≥7 mmol/dl
Han et al. (29) (1,244/86/6)	C-reactive protein	Age, smoking, alcohol, physical activity	OR (3Q vs. 1Q) = 5.4 (2.2–13.4)	Associations for women only (729/54)
Vozarova et al. (20) (272/54/5)	White blood cell count	Age, sex, percentage of body fat, acute insulin response, insulin sensitivity (clamp)	HR (90th vs. 10th) = 2.6 (1.1–6.2)	Subjects with normal glucose tolerance at baseline
Duncan et al. (26)	IL-6 (C-reactive protein, orosomucoid, sialic acid)	Age, sex, center, ethnicity, blood pressure, familial history of diabetes, fasting glucose, fasting insulin, waist-to-hip ratio, BMI	HR (4Q vs. 1Q) = 1.6 (1–2.7)	Cohort random sample 1,153 case/control (581/572)
Nakanishi et al. (36) (947/122/6)	C-reactive protein	Age, smoking, familial history of diabetes, normal glucose tolerance/impaired glucose tolerance, BMI, HOMA, hormone replacement therapy (women)	HR (4Q vs. 1Q) = men 2.84 (1.09–7.39) women 3.11 (1.25–7.75)	Men (396/57) women (551/65); in men if C-reactive protein used as continuous variable HR = NS
Spranger et al. (33)	C-reactive protein, IL-6 (IL-1β, TNF-α)	Age, sex, center, smoking, alcohol, physical activity, education, BMI, waist-to-hip ratio, HbA_{1c}	C-reactive protein: OR 1.9 (1.2–3.2) IL-6: OR (4Q vs. 1Q) = 2.6 (1.2–5.5)	Nested case (188) control (377) study; stronger HR if HbA_{1c} <5.8%

Thorand et al (34) (2,052/101/7)	(C-reactive protein)	Age, survey, BMI	HR (4Q vs. 1Q) = 1.9 (0.97–3.6)	C-reactive protein does not predict diabetes independently of BMI
Hu et al. (30) (32,826/737/10)	C-reactive protein (TNF-α receptor 2, IL-6)	Age, fasting status, race, smoking, physical activity, familial history of diabetes, hormone replacement therapy, menopause, diet score, BMI, inflammatory markers	OR (5Q vs. 1Q) = 3.99 (P for trend < 0.001)	C-reactive protein showed similar associations if e-selectin, HbA_{1c}, fasting insulin, waist-to-hip ratio, and removing of incident cardiovascular disease cases included in the model
Laaksonen et al. (35) (762/78/11)	C-reactive protein	Age, cardiovascular disease, physical activity, socioeconomic factors, alcohol, smoking, familial history of diabetes, waist-to-hip ratio, fasting glucose, fasting insulin, triglycerides, hypertension	OR (3Q vs. 0.1Q) = 2.3 (1.04–5.07)	Inclusion of BMI in the model does not affect results

*Inflammatory markers in parenthesis were not independently related to risk of type 2 diabetes after adjustment for confounders. 3Q vs. 1Q, comparison of tertiles; 4Q vs. 1Q, comparison of quartiles; 5Q vs. 1Q, comparison of quintiles; 90th vs. 10th, comparison of percentiles; CTD, converters to diabetes; FU, follow-up time; HOMA, homeostasis model assessment; HR, hazard ratio; IRR, incidence rate ratio; OR, odds ratio; RR, relative risk.

studies in which substantial baseline differences in the degree of adiposity exist, the predictive value of inflammatory markers may be a result of their association with obesity, i.e., they may be acting as surrogate markers of hypoadiponectinemia and may be only indirectly associated with the development of diabetes (Fig. 1*B*) (Table 2).

Adipose tissue is also a source of other anti-inflammatory cytokines whose roles are mostly unknown. IL-10 is an anti-inflammatory cytokine produced by immune cells (T-helper, B-cells, and macrophages). In mice, IL-10 treatment prevented IL-6–induced defects in both hepatic and skeletal insulin action (56). Circulating IL-10 is elevated in human obesity (80); in adipose tissue, IL-10 is primarily produced by nonfat cells (81). In humans, lower serum concentrations of IL-10 were associated with the metabolic syndrome (80) and with diabetes (82).

CHRONIC ACTIVATION OF THE INNATE IMMUNE SYSTEM AND IMPAIRMENT OF INSULIN SIGNALING AND/OR INSULIN SECRETION: PUTATIVE MOLECULAR MECHANISMS

Since it was first proposed that chronic activation of the innate immune system could be a possible pathogenic factor in the development of obesity-associated type 2 diabetes, the challenge has been to identify the molecular link between these two entities. The innate immune response and the process of inflammation are inextricably interwoven. Signaling receptors of the immune system (such as the mammalian toll-like receptors) induce signal transduction pathways that lead to the activation of transcription factors (83) that are also activated in response to proinflammatory cytokines. At this point, the leading theory is that activation of some inflammatory pathways, by some of the adipokines previously discussed, ultimately results in suppression of the insulin signal transduction by serine/threonine (Ser/Thr) phosphorylation (inactivation) of the IRS. Two major inflammatory transcription factors, NF-κB and activating protein (AP)-1, and their key enzymes IκB kinase (IKK) and c-Jun NH_2-terminal kinase (JNK), respectively, have been studied more extensively. However, obesity may cause activation of the innate immune/inflammatory system not only by its secreted adipokines. Hyperlipidemia in mice seems to mediate an inflammatory response by the same signaling cascade (engaged by a receptor complex comprising mammalian toll-like receptor 4, CD14, and MD-2) through which lipopolysaccharide activates the innate immune system (84). FFAs, probably through protein kinase C, can activate IKK and JNK (85). Furthermore, oxidative stress (closely associated with obesity, hyperglycemia, and elevation of FFAs) not only leads to mitochondrial dysfunction but can also induce key redox-sensitive transcription factors (NF-κB and AP-1) involved in the innate immune response.

Genetic disruption of these pathways improves insulin resistance (86,87). Heterozygous $IKK\beta^{+/-}$ mice, fed with a high-fat diet or crossed with obese *ob/ob* mice, showed a significant decrease in blood glucose levels and improved insulin resistance (87). Furthermore, lipid infusion–induced decreases in insulin-stimulated tyrosine phosphorylation of IRS-1 and IRS-1–associated PI 3-kinase activity in skeletal muscle were prevented in the $IKK\beta^{+/-}$ mice (88).

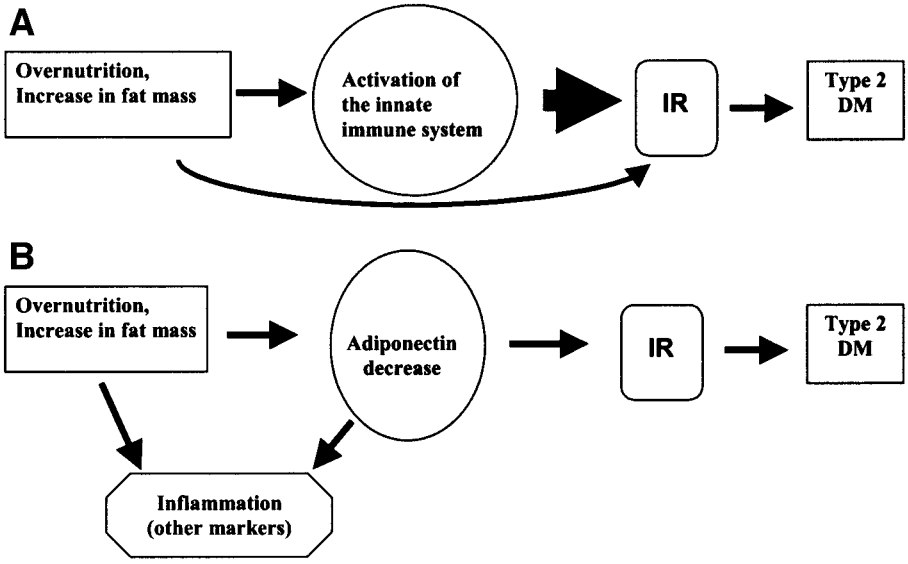

FIG. 1. ***A*****: Overnutrition and obesity cause an activation of the immune system, which in turn promotes insulin resistance, ultimately leading to type 2 diabetes. Lipotoxicity, glucotoxicity, cellular nutrient overload, and genetic predisposition are other pathophysiological mechinisms through which obesity leads to insulin resistance.** ***B*****: As proposed by Krakoff et al. (79), the predictive value of inflammatory markers may be a result of their association with obesity, i.e., they may be acting as surrogate markers of hypoadiponectinemia and may be only indirectly associated with the development of diabetes.**

Recently, this same laboratory has found that selective IKKβ activation (transgenic mice) in fat or liver but not muscle causes systemic insulin resistance. In agreement with these results, selective inhibition of NF-κB (by expressing IκBα super-repressor) in fat (FISR) and liver, but not in muscle, showed protection against the development of insulin resistance in diet-induced and genetically obese mice, with the additional benefit of preventing weight gain in FISR mice (89)

Total JNK activity, predominantly JNK1, is increased in obese mice (86); JNK1 knockout mice gain less weight and are protected against diet-induced insulin resistance or insulin resistance associated with a genetic model of obesity (*ob/ob*) (86). It appears that in these mice, serine-307 phosphorylation of IRS-1 was reduced (86). Moreover, suppression of the JNK pathway in liver decreases whole-body insulin resistance and improves glucose tolerance in diabetic animal models (90). Furthermore, loss-of-function mutations in JNK-interacting protein 1, a negative and essential modulator of JNK (91), causes type 2 diabetes in humans (92).

Additional evidence of the involvement of these inflammatory pathways is supported by the protective effect of some anti-inflammatory drugs against obesity-induced insulin resistance. Aspirin may inhibit not only IKK and JNK (93,94) but other Ser/Thr kinases (mamalian target of rapamycin and protein kinase B/Akt) related to TNF-α insulin resistance by phosphorylation of IRS-1 at serine residues (94). Furthermore, through its antioxidant properties, aspirin has been shown to reduce the activation of NF-κB or AP-1 associated with reactive oxygen species (95). Yuan et al. (87) first hypothesized and then demonstrated that salicylate treatment improves the severe insulin resistance seen in genetically obese rodents. Furthermore, pretreatment in rats with salicylates prevented lipid-induced skeletal insulin resistance by inhibiting lipid-induced decreases in insulin-stimulated IRS-1 tyrosine phosphorylation and IRS-1–associated PI 3-kinase activation (88). In humans, treatment with high doses of aspirin (7 g/day) or salsalate (3 g/day) improved peripheral insulin sensitivity in subjects with type 2 diabetes (96,97). Although these results are intriguing, randomized controlled

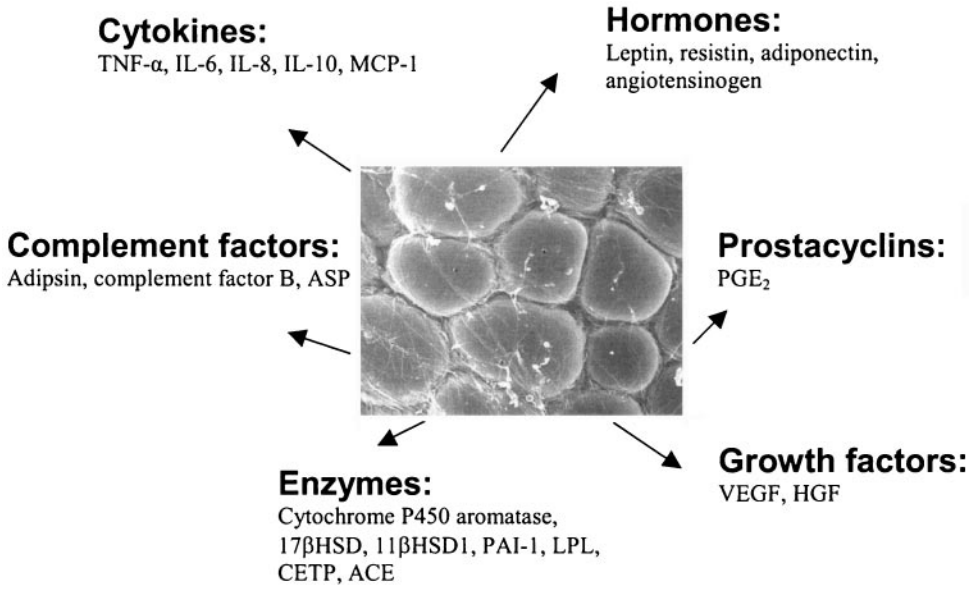

FIG. 2. Adipose tissue–derived proteins. Adipose tissue secretes a number of proteins with different functions. Enzymes are involved in steroid (cytocromo P450 aromatase, 17β-hydroxysteroid dehydrogenase [17βHSD], and 11β-hydroxysteroid dehydrogenase [11βHSD]) and lipid (lipoprotein lipase [LPL], cholesterol ester transfer protein [CETP]) metabolism, fibrinolitic system (plasmonigen activator inhibitor-1 [PAI-1]), and blood pressure regulation (ACE). ASP, acylation-stimulating protein; HGF, hepatic growth factor; MCP-1, monocyte chemoattractant protein-1; PGE_2, prostaglandin E2; VEGF, vascular endothelial growth factor.

TABLE 2
Effect of adiponectinemia on risk of type 2 diabetes: prospective studies

Authors (ref.) (*n*/CTD/FU)	Inflammatory markers*	Confounders accounted for in final model	Risk of developing type 2 diabetes (95% CI)	Comments (*n*)
Krakoff et al. (79)	Adiponectin (C-reactive protein, IL-6, TNF-α, secretory phospholipase A2, von Willebrand factor, soluble intercellular adhesion molecule-1, endothelial markers E-selectin, soluble vascular cell adhesion molecule-1)	Age, BMI, sex, fasting glucose, fasting insulin, 2-h glucose, HbA_{1c}, waist	IRR 0.63 (0.41–0.98)	Case/control (71/71) nested study; OR calculated for 1 SD difference
Daimon et al. (74) (978/54/5)	Adiponectin (TNF-α)	Age, sex, waist-to-hip ratio, 2-h glucose, TNF-α	OR 0.766 (P = 0.029)	Normal glucose tolerance (837) to diabetes (18); similar results in impaired glucose tolerance to diabetes; OR calculated per 0.1 log μg/ml difference
Spranger et al. (78)	Adiponectin	Age, sex, BMI, waist-to-hip ratio, HbA_{1c}	OR 0.90 (0.84–0.97)	Case/control (187/376) study; sensitive analysis with HbA_{1c} <6%; subjects showed no differences
Snehalatha et al. (77) (91/25/1)	Adiponectin	Age, sex, BMI, waist, percentage of body fat, HbA_{1c}, HOMA-IR, fasting glucose, 2-h glucose	OR 0.87 (0.79–0.95)	Impaired glucose tolerance subjects at baseline
Duncan et al. (75)	Adiponectin	Age, sex, center, ethnicity, familial history of diabetes, fasting glucose, fasting insulin, waist-to-hip ratio, BMI, inflammatory score	HR (4Q vs. 1Q) = 0.58 (0.34–0.99)	Cohort random sample 1,153 case/control (581/572) study
	Adiponectin, white blood cell count, sialic acid (C-reactive protein, fibrinogen, orosomucoi, IL-6)	Age, sex, center, ethnicity, familial history of diabetes, fasting glucose, fasting insulin, waist-to-hip ratio, BMI, all six markers	Adiponectin: HR 0.56 (0.33–0.96); white blood cell count: HR 1.66 (1.04–2.63); sialic acid: HR 1.73 (1.08–2.78)	In smokers, adiponectin does not protect against diabetes

*Inflammatory markers in parenthesis were not independently related to risk of type 2 diabetes after adjustment for confounders. 3Q vs. 1Q, comparison of tertiles; 4Q vs. 1Q, comparison of quartiles; 5Q vs. 1Q, comparison of quintiles; 90th vs. 10th, comparison of percentiles; CTD, converters to diabetes; FU, follow-up time; HOMA-IR, homeostasis model assessment of insulin resistance; HR, hazard ratio; IRR, incidence rate ratio; OR, odds ratio; RR, relative risk.

trials are needed to clarify the role of these drugs on glucose metabolism and insulin resistance.

Other drugs with documented anti-inflammatory effects, such as thiazolidinediones (TZDs) and statins, have shown antidiabetic effects. TZDs are potent insulin sensitizers, and TZD treatment has been associated with suppression of local TNF-α production by adipocytes and reduction of TNF-α action in adipose and other tissues (98). TZDs can also act by increasing plasma levels of adiponectin, and some studies have suggested that peroxisome proliferator–activated receptor γ (like peroxisome proliferator–activated receptor α) activation in selected cell types can repress NF-κB and, therefore, cytokine-mediated signaling (98). Statins are potent cholesterol-lowering drugs. Statin treatment has been associated with modulation in the endothelial adhesion and transendothelial migration of leukocytes, inhibition of the release of cytokines, and chemokines and direct interference with the NF-κB pathway (99). Interestingly, the use of statins has also been associated with a decrease in the risk of developing type 2 diabetes (100).

We have discussed how activation of protein kinases and transcription factors, such as AP-1 and NF-κB, are possible mediators of insulin resistance in peripheral tissues. Because the same molecules are directly involved in β-cell apoptosis (101), it is possible that an adipokine-induced activation of the immune system associated with overnutrition and obesity may also explain the β-cell failure that precedes the development of type 2 diabetes. Here, data from the literature do not paint a very clear picture. For example, adiponectin has been shown in vitro to have protective effects against both cytokine- and FFA-induced impairment of the β-cell (102), an effect that would be lost in obese individuals with hypoadiponectinemia. Leptin, which is very high in obese individuals, has been shown in vitro to have both stimulatory and inhibitory effects on β-cell apoptosis (103). More important, thus far there are no reports in the literature from in vivo animal or human studies of an independent association between (markers of) inflammation and β-cell dysfunction.

IS EXCESSIVE FATNESS AN OBLIGATORY PATHOPHYSIOLOGICAL FACTOR?

While we have developed this review around the pathophysiological construct that overnutrition leads to obesity which in turn is associated with a chronic activation of the immune system, we would like to acknowledge experimental and circumstantial evidence that challenges this course of events.

The postprandial period following a single meal is associated with an increase in plasma levels of proinflammatory cytokines, recruitment of neutrophils, and oxidative stress (104–106). The quality, intensity, and duration of this inflammatory response may not only respond to frequency or meal size. The effect of nutrition or food-borne components on gene transcription, proteomics, and metabolism (nutrigenomic) may further increase this inflammatory state. Because modern eating patterns, especially in western societies, produce an almost endless postprandial state throughout the day, a chronic activation of the innate immune system could exist even before obesity develops. Thus, a proinflammatory state could be a pathogenic factor in the development of obesity, as proposed by Das (107) and others. Consistent with this hypothesis, two prospective studies (108,109) reported that elevated levels of inflammatory markers predicted weight gain in two different populations. Finally, a recent study (110) showed that removal of significant amounts of subcutaneous fat tissue had no effect on inflammation markers or insulin sensitivity.

Thus, contrary to evidence presented earlier in the manuscript, it is possible that it is not the mass of the adipose tissue per se but the underlying changes in energy flux that determine its size that is responsible for the modulation of the innate immune response. However, the adipose tissue is not a homogeneous tissue, and characteristics between depots and even between cells in different parts of the same depot differ within and between individuals. Of particular interest to this discussion is the idea that perivascular fat tissue may be especially detrimental in obese people. Increased perivascular fat may not only contribute to the systemic low-grade inflammation associated with obesity but may also interact in an autocrine/paracrine manner, with the closely related endothelial cells and perivascular smooth muscle fibers contributing to the endothelial dysfunction that has often been observed in association with markers of inflammation. Whereas impaired endothelial dysfunction in large arterial beds may have an influence on the pathogenesis of cardiovascular disease, endothelial dysfunction in arteriole and capillaries, in intimate contact with a vast surface of metabolically active and insulin-sensitive tissues, may lead to type 2 diabetes (111,112). This is obviously an area that will require further research.

CONCLUSIONS

Does an adipokine-induced activation of the immune system mediate the effect of overnutrition on type 2 diabetes? We interpret the literature reviewed in this manuscript as indicating that a reasonable case can be made for overnutrition causing an activation of the innate immune system, most likely by excessive production of adipokines. There is increasing evidence that the proinflammatory state, which characterizes overnourished individuals, may be etiologically linked with the insulin resistance that is often observed in their peripheral tissues. Surprisingly, we could not find convincing evidence for an association between inflammation and insulin secretory dysfunction.

Thus, we propose that inflammation should be viewed as a risk factor for insulin resistance not a global risk factor for type 2 diabetes. In our opinion, the question of how β-cells fail in the presence of inflammation-induced insulin resistance remains largely unanswered. Moreover, this may help explain why diabetes does not develop in all subjects with chronic inflammatory diseases.

Notwithstanding these considerations, we believe that elucidation of the link between inflammation and insulin resistance remains a very worthwhile research endeavor. We and others have provided evidence suggesting that interindividual variability of many of these inflammatory markers may be genetically determined. It has been hypothesized that an insulin-resistant genotype, associated with a heightened cytokine response, may have been advantageous in the historical conditions of a short life span, injury, and infectious disease (113). A further selec-

tion for these traits may have taken place in American Indians and other native populations after first contact with explorers from other parts of the world, which exposed them to a range of novel infectious diseases to which they had no immunity, leading to repeated epidemics and declines in population. It will be interesting to see if this theory is confirmed when the results of ongoing positional cloning efforts to find the genes that cause diabetes in several populations around the world become available.

In more practical terms, more fully understanding the link between inflammation and insulin resistance holds the promise of revealing novel ways to prevent and/or treat type 2 diabetes. If it can be demonstrated that reducing the underlying activity of the immune system in nondiabetic subjects with high markers of inflammation can improve their degree of insulin sensitivity, then perhaps a case can be made for anti-inflammatory therapy as a way to prevent and/or delay the onset of type 2 diabetes. Early clinical trials are showing promising results when anti-inflammatory drugs are given to diabetic subjects. This indicates that as the chain of molecular events linking inflammation to impairment of insulin signaling will continue to be clarified, novel targets will become available for drug development. Thus, we see many reasons to be optimistic and expect that the question of the link between inflammation, insulin resistance, and type 2 diabetes may no longer be a burning one in the not so distant future.

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