The Development of Porcine Models of Obesity and the Metabolic Syndrome[1,2]

Michael E. Spurlock* and Nicholas K. Gabler

Department of Food Science and Human Nutrition, Iowa State University, Ames, IA 50011

Abstract

Despite aggressive research aimed at understanding the myriad biochemical factors that are integrated to balance energy intake and expenditure to maintain normal body weight, obesity is increasing at an alarming rate, and the long-term success of prevention and intervention strategies is minimal. Because much of the scientific literature addressing obesity has originated with rodent models, there is considerable interest among researchers and funding agencies in the development of comparative animal models. Furthermore, numerous disparate results between rodent models and humans (i.e., adipsin, leptin, resistin, tumor necrosis factor-α, and other adipokines) have hindered the translation of rodent data into actionable technologies for humans. The pig is an exceptional restenosis model, and is emerging rapidly as a biomedical model for energy metabolism and obesity in humans because it is devoid of brown fat postnatally and because of their similar metabolic features, cardiovascular systems, and proportional organ sizes. This article highlights the current literature devoted to the development of porcine models for obesity and the metabolic syndrome, with a particular emphasis on the role of adipose tissue and adipokines in the regulation of energy balance and the inflammation associated with obesity.   J. Nutr. 138: 397–402, 2008.

Introduction

Obesity is generally recognized as a chronic disease defined by an overaccumulation of fat stores in adipocytes and is frequently linked with inflammation in adipose tissue and insulin resistance in peripheral tissues (1,2). Moreover, the common pathogenesis of type II diabetes involves a genetic predisposition to obesity (i.e., a thrifty genotype) and a gradual diminution of insulin sensitivity in a prediabetic condition termed the metabolic or cardiometabolic syndrome (3,4). To date, obesity and the associated chronic inflammation and insulin resistance are among the most prevalent diseases in developed countries and impose enormous health detriments and economic burdens on the U.S. and global economies (5). The costs associated with diabetes are estimated at some $100 billion annually in the United States, and the losses in quality of life and shorter life expectancies are devastating.

Recent studies and predictions indicate that the prevalence of obesity in adults increased from 13 to 32% between the 1960s and 2004, and that 66% of adults and 16% of children are at present overweight or obese; based on current increases in childhood obesity, predictions are that 75% of adults will be overweight or obese by 2015 (6). These numbers and predictions are of great concern to government officials, researchers, and the general public, and this concern is only exacerbated by the limited success achieved to date by the research community with respect to long-term prevention and intervention strategies for obese or obesity-prone individuals.

Despite very aggressive research agendas aimed at defining the molecular basis of obesity and its comorbidities in humans, the underlying and integrated biochemical mechanisms have not been clearly identified. Rodent models have been the pillar of obesity and metabolic syndrome research because they are inexpensive to maintain, have a sequenced genome, and are easily modified by genetic engineering. Albeit, apart from the Zucker rat, which is extremely hyperphagic because of the absence of a functional leptin receptor, rodent models that reliably develop 3 or more of the clustered risk factors required for a consensus declaration of the metabolic syndrome are lacking, and other animal models consistently showing 4 or more risk factors have not been identified. Furthermore, there are clear metabolic and physiological differences between humans and rodents, and these differences have undoubtedly slowed progress and complicated the translation of biomedical research findings into effective preventive or intervention therapies for obesity and its comorbidities. As summarized in **Table 1**, researchers are now recognizing that marked differences in metabolism and adipose tissue biology between rodents and humans have hindered the translation of research findings into effective prevention and intervention technologies and strategies for alleviating the human crisis (7). Consequently, alternative and complementary models are being pursued, and the pig is

TABLE 1 Rodent and human adipokines illustrating important differences related to obesity, insulin resistance, and inflammation[1]

Adipokine	Mouse	Human
Adipsin	Lower in obese	Same or higher in obese
TNFα	Neutralization improves insulin sensitivity	Neutralization has no effect on insulin sensitivity
Resistin	Impairs glucose tolerance and insulin sensitivity	Levels do not reflect insulin resistance or obesity

[1] Summarized from Arner (7).

emerging as an attractive biomedical model for energy metabolism and obesity in humans because it is devoid of brown fat postnatally, as are humans. This is an important consideration because of the ability of brown fat to regulate energy balance and other aspects of energy homeostasis. The pig also has similar metabolic features and cardiovascular system and proportionally similar organ sizes. Furthermore, adipose depots in pigs are of sufficient size that multiple assays can be carried out on adipocytes or stromal vascular cells without pooling across depots or animals. This article highlights the current status of the pig as an obesity model, our understanding of inflammatory pathways in porcine adipocytes and adipose tissue, and identifies key areas requiring further development.

Insulin resistance and atherosclerosis in current porcine obesity models

Although some metabolic and cardiovascular disorders occur in domestic swine partially as a result of intense selection for growth rate and leanness (8), attempts to induce frank type II diabetes or even sufficient criteria for a consensus diagnosis of the metabolic syndrome have met with limited success. However, the contemporary domestic pig, when fed substantial quantities of lard and cholesterol becomes an excellent humanoid model for atherosclerosis, and researchers have quite successfully extended this model to include atherosclerosis accelerated by streptozotocin-induced diabetes (9). Furthermore, contemporary pigs fed a Paleolithic diet consistent with the hunter-gather lifestyle of our ancestors are leaner, more sensitive to insulin, and have lower circulating concentrations of C-reactive protein than their counterparts fed a cereal diet reflective of modern-day habits (10). However, the Gottingen, Yucatan, and Ossabaw breeds of swine have been used more extensively for investigations of obesity and cardiovascular disease. Among these, the Ossabaw breed appears to be a particularly valuable model. As noted in Brisbin's defense of feral pig populations as exceptional contributors to global biodiversity (11), the current speculation is that these swine have undergone natural selection for the thrifty genotype to survive seasonal cycles of feasting and famine during their 500 y of isolation on the Ossabaw Island located off the coast of Georgia, USA. Ossabaw swine allowed to eat excess food in captivity have the highest levels of total body lipid of any mammal and become morbidly obese even in the absence of a high-fat diet (**Fig. 1**). As summarized in **Table 2**, it is even more compelling that female Ossabaw swine fed a high-fat cholesterol diet develop at least 5 of the 6 criteria of the metabolic syndrome, including primary insulin resistance, obesity with significant visceral adipose expansion, hypertriglyceridemia and increased LDL:HDL cholesterol, mild hypertension, and coronary artery disease (12). Primary insulin resistance has been difficult to achieve in other swine models (13), and because both primary insulin resistance and coronary disease develop in the Ossabaw female, this pig may be an extraordinary model that will enable a greater understanding of why the frequency of the metabolic syndrome and death related to coronary heart disease are increasing at such an alarming rate in women (12). Based on these recent findings in this novel model, there is indeed a compelling need to understand the relations among adipose depots, adipokine biology, and the onset and progression of the constellation of factors that comprise the metabolic syndrome and mark the transition to frank diabetes.

Porcine adipocytes express functional TLR-4

Chronic inflammation is a significant contributor to the development of obesity-linked insulin resistance, and biomedical models will of necessity need to recapitulate this chronic inflammation. Hallmarks of the chronic inflammatory state include increased adipose expression of tumor necrosis factor (TNF)-α and interleukin (IL)-6, and increased circulating concentrations of IL-6 (14,15). Additionally, the immunological implications for adipocytes in relation to obesity are expanding rapidly. Experiments performed with cultured 3T3-L1 adipocytes indicate that adipocytes express Toll-like receptors (TLR)[3] and respond to bacterial lipopolysaccharide (LPS) by producing TNFα and IL-6, classical proinflammatory cytokines (16). We have extended these findings to include primary pig adipocytes and determined that stimulation of these cells with LPS invokes activation and nuclear translocation of the nuclear factor-κB (NFκB) transcription factor (17). IL-6 is more highly expressed under basal conditions than is TNFα and is more responsive to LPS in terms of the accumulated concentration of this cytokine in stimulated cells than is TNFα (17). Additionally, IL-6 normally circulates at significant concentrations in pigs, whereas TNFα is typically quite low (18,19). This concentration difference parallels the typical human scenario and renders the regulation of IL-6 production and signaling in pig adipocytes of considerable importance in the development and characterization of effective models of obesity and the metabolic syndrome.

Inflammation in porcine adipose tissue

Recent publications with rodent models have established strong linkages between TLR-4 and the onset and progression of obesity and the associated inflammation; saturated fatty acids mediate their proinflammatory effects, at least in part, via TLR-4 activation (20), and activation of this receptor either by endotoxin of intestinal origin or dietary fat induces an obesity response (21). As yet, no studies have addressed the acute effects of saturated fatty acids on inflammation in primary pig adipocytes or in those derived from cultured stromal vascular cells. It also remains to be determined whether dietary fat intake and fatty acid profiles alter circulating endotoxin concentrations in porcine adipocytes and whether these changes relate to similar events in humans. Nonetheless, we have recently shown that

---

[3] Abbreviations used: ACC, acetyl-CoA carboxylase; AMPK, AMP-activated kinase; CCR2, chemokine receptor 2; IL, interleukin; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; nuclear factor, NF; TLR, Toll-like receptor; TNF, tumor necrosis factor.
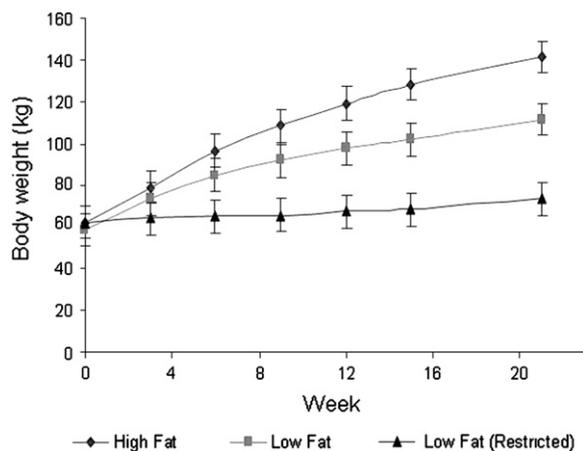
FIGURE 1 Development of obesity in Ossabaw swine allowed unrestricted consumption of a high-carbohydrate diet devoid of added fat, or a diet high in saturated fat. The lean control consumed the same diet as the low-fat group, but intake was restricted to maintain lean mature body weight. All 3 groups were significantly different from each other by wk 6 ($P < 0.01$). Consistent with the overconsumption driven by the thrifty genotype, the pigs fed the energy-dense high-fat diet did not reduce intake (data not shown).

dietary fatty acid profiles do indeed influence the ability of exogenous endotoxin to down-regulate the TLR-4 receptor protein in porcine adipose tissue (22). Thus, the pig may provide, at minimum, a valuable comparative model for establishing and comparing the roles of fatty acids on inflammatory pathways in relation to obesity, and with availability of the porcine genome sequence in the near future (23), detailed analyses of TLR-4 alleles in relation to obesity and the metabolic syndrome may prove quite informative.

There is now compelling evidence that obesity results in a marked accumulation of macrophages in the adipose tissue and transcript profiles in adipocytes and macrophages that reflect active inflammatory states (24–27). The chemokines monocyte chemoattractant protein-1 (MCP-1), macrophage migration inhibitory factor, and macrophage inflammatory protein-1α, are likely contributors to the infiltration of adipose tissue with immunocytes. MCP-1 expression in adipose tissue is markedly increased with obesity in rodent models (28,29), and Xu et al. (24) showed not only increased expression of MCP-1 in the adipose stromal vascular cells, but similar results for macrophage inflammatory protein. Chen et al. (30) found that a high-fat diet induces the expression of MCP-1 and MCP-3 in rodent adipose tissue, and MCP-1 is clearly induced by IL-6 in 3T3-L1 adipocytes (31,32). This is of considerable interest for 2 reasons.

TABLE 2 Comparison of Ossabaw and Yucatan swine as a model of the human metabolic syndrome.

Criterion	Yucatan	Ossabaw
Obesity	No	Yes
Insulin resistance[1]	No	Yes
Glucose intolerance	No	Yes
Dyslipidemia[2]	Yes	Yes
Dyslipidemia[3]	No	Yes
Hypertension	No	Yes

[1] Primary insulin resistance.
[2] Increased LDL:HDL cholesterol with no change or a reduction in HDL concentration.
[3] Increased triglyceride.

First, IL-6 is elevated in the circulation of obese humans (32) and Ossabaw swine (13), and second, a substantial amount of the IL-6 expression in adipose tissue originates in adipocytes rather than stromal vascular cells (25). Consequently, if a high-fat diet induces an inflammatory response in adipocytes that includes the production of IL-6, as is certainly suggested by our recent study (33), then dietary fat is likely an intimate determinant of macrophage recruitment and activation in adipose tissue. Weisberg et al. (25) have shown a critical role for the chemokine receptor 2 (CCR2), to which MCP-1 binds with high affinity, in macrophage recruitment and inflammation in adipose tissue. In this important study, genetic deficiency of CCR2 attenuated the development of obesity and reduced the macrophage content and inflammatory markers in adipose tissue versus controls matched for adiposity. Whereas others have obtained contradictory results (34), Neels and Olefsky (35) have pointed out the likelihood that duration of high-fat feeding and the genetic background on which the CCR2 deficiency was introduced were potential factors. These types of contradictions underscore the need for comparative models. However, we are not aware of any study in which obesity-linked macrophage infiltration in adipose tissue has been reported for the pig, nor are we aware of any comparative mechanistic data.

Adiponectin in pigs
Adipokines are regulatory molecules secreted by adipose tissue, and apart from those noted above, adiponectin seems to be intimately linked to obesity and inflammation in pigs, as it is in humans. Fruebis et al. (36) provided compelling evidence that adiponectin regulates lipid metabolism and body weight in rodents. Additional work from others (37) showed that adiponectin actually stimulates glucose transport in primary rat adipocytes via a mechanism that is independent of tyrosine phosphorylation of the insulin receptor and activation of IRS-1. Insulin-stimulated glucose uptake was enhanced when the insulin concentration was less than that required to maximize glucose transport. These effects were dependent on activation of the AMP-activated kinase (AMPK) and occurred with a parallel phosphorylation (deactivation) of acetyl-CoA carboxylase (ACC). We have now shown that adiponectin suppresses the incorporation of glucose carbon into lipid in primary pig adipocytes (17), a response consistent with the deactivation of ACC by adiponectin's activation of AMPK.

Early work with adiponectin indicated that circulating concentrations are reduced in association with obesity and insulin resistance (38). Since then, others have confirmed these findings in different ethnic groups (39–41) and also showed clear relationships between hypoadiponectinemia and dyslipidemias (42–44). We have shown in pigs that adiponectin and leptin are reciprocally regulated with obesity, as they are in humans; whereas serum leptin and adipose mRNA expression increase with adiposity in pigs, adiponectin is considerably lower in fatter genotypes (17). This is an important observation because in the fa/fa rat model, increased expression of adiponectin in brown adipose tissue seems to negate the reduction in white adipose tissue in some circumstances. Thus, the circulating adiponectin concentration can actually be increased in this obese rat versus the lean controls (45). There is a growing body of literature that indicates that the hypoadiponectinemia of obesity is explained largely by a reduction in adiponectin expression in visceral adipocytes versus subcutaneous adipocytes, at least in Japanese men (46) and Zucker fatty rats (47). This concept has been underscored in humans in that omental adipocytes produced more adiponectin in vitro (per unit of DNA) and also responded

more vigorously to insulin, rosiglitizone, or the combination of insulin and rosiglitizone than did subcutaneous adipocytes. Fasting plasma insulin concentrations are negatively correlated with plasma adiponectin concentrations, and chronic treatment of 3T3-L1 adipocytes with insulin reduces adiponectin mRNA expression (48–50). However, others have found insulin to increase adiponectin mRNA expression in human visceral adipocytes and mouse brown adipocytes (51,52), and it is quite possible that there is differential regulation of adiponectin across species and perhaps across adipose tissue depots. These possibilities must be addressed experimentally in pigs as model development moves forward.

Because adiponectin is produced largely by the adipocyte, the antiinflammatory activity of this adipokine may be particularly important to the chronic inflammatory state in adipose tissue that is common to obesity. Yokota et al. (53) first reported that adiponectin suppressed proinflammatory cytokine production in activated human macrophages, and since then, we have obtained similar results for both TNF and IL-6 in activated pig macrophages (54) and THP-1 monocytes (55). We have also extended this concept to porcine adipocytes in that NFκB activation is disrupted and IL-6 expression and release are attenuated by adiponectin when inflammation is induced by LPS (56).

Proinflammatory cytokines that are strongly associated with the development of insulin resistance also down-regulate adiponectin expression. Both TNF and IL-6 inhibit adiponectin mRNA expression or protein synthesis in 3T3-L1 or human adipocyte or both (57–60). Recent confirmation of the negative effect of IL-6 was provided when Sopasakis et al. (61) found a higher concentration of IL-6 in the interstitial fluid than in plasma and then showed that adipocytes exposed to concentrations of IL-6 comparable to that in the interstitial fluid had lower adiponectin mRNA abundance. Thus, it seems that the induction of an inflammatory response would suppress adiponectin, and because of the antiinflammatory activity of this hormone, the inflammatory response would likely be amplified by the decline in adiponectin. This cycle is perhaps central to the progression of the inflammation associated with obesity, and there is considerable need to understand the relation among adiponectin status, obesity, and inflammation in the adipose depots of porcine obesity models.

The regulation of AMPK by adiponectin may be a critical determinant of energy metabolism and storage, and indeed, AMPK has received a great deal of attention with respect to obesity and its comorbidities. The AMPK complex, in most eukaryotic species, is a well-conserved serine/threonine kinase heterodimer consisting of α-, β-, and γ-subunits (62). These AMPK subunits act as fuel gauges and are activated by any stress that depletes cellular ATP with a reciprocal rise in AMP. This enzyme not only promotes glucose uptake through glut-4 (63,64) but also promotes fatty acid oxidation (65–68).

Interestingly, in the mid-1980s the identification and characterization of an AMPK γ3 mutation in pigs started with the discovery of the RN gene (69). Common to the Hampshire breed, the major phenotypic result of this mutation is a marked increase (~70%) in skeletal muscle glycogen content, which results in meat with a lower ultimate pH and water content (70). However, liver and heart glycogen content is unchanged. This RN pig phenotype is caused by a single missense mutation ($Arg^{200} \rightarrow Gln$, R225Q) in the protein kinase AMP-activated γ3 gene, PRKAG3, which encodes the γ3 isoform of AMPK (71). Additionally, a second mutation (V224I) was identified in pig muscle on the third functional allele at the PRKAG3 locus, caused by a $Val^{199} \rightarrow I1e$ missense mutation (72). This mutation

is associated with an opposite effect, low glycogen and high pH, compared with the RN allele. Thus, R225Q is a loss-of-function mutation that abolishes allosteric regulation by AMP/ATP, causing increased AMPK activity compared with V224I.

Although there is no direct evidence in pigs, the R225Q mutation may provide protection against the development of insulin resistance and Type II diabetes by increasing lipid oxidation. Shen et al. (73) hypothesized that decreased AMPK activity is associated with the missense mutation V224I in obese Ossabaw pigs (74), leading to altered skeletal muscle AMPK activity and causing increased mammalian target of Rapamycin (mTOR) activity. These authors also speculated that negative feedback by mTOR-p70S6k signaling on IRS-1 may be causal in the development of insulin resistance in obese swine, but this is as yet unresolved. An additional implication for AMPK and adiponectin in porcine models encompasses liver lipid metabolism. Using Yucatan minipigs fed folate-deficient diets and 40% ethanol, others have shown increased hepatic lipid synthesis in association with down-regulated adiponectin-mediated pathways driven by activated AMPK (75). As noted previously, it is well documented that adiponectin stimulates glucose utilization and fatty acid oxidation by activating AMPK (76), but our understanding of these pathways in porcine obesity models is in its infancy, and whether these relationships accurately reflect human biology is not yet known.

The obesity crisis is the major health issue facing public and private research entities and government agencies, and whereas rodent models have led to many significant and meaningful discoveries, it is clear that alternative and complementary models will expedite the translation of science into actionable technologies for prevention and intervention. The pig, and the Ossabaw female in particular, may play an integral role in defining the molecular basis of obesity and its comorbidities because virtually all of the hallmark criteria of the metabolic syndrome develop in this animal in a relatively short time span. This, coupled with the porcine genome sequence and an ever increasing availability of molecular reagents for the pig, will provide researchers a stronger platform from which to pursue novel strategies to alleviate this tremendous health detriment.

Literature Cited

1. Bray GA. Obesity: the disease. J Med Chem. 2006;49:4001–7.

2. Bray GA, Bellanger T. Epidemiology, trends, and morbidities of obesity and the metabolic syndrome. Endocrine. 2006;29:109–17.

3. Cheung BM, Ong KL, Man YB, Wong LY, Lau CP, Lam KS. Prevalence of the metabolic syndrome in the United States National Health and Nutrition Examination Survey 1999–2002 according to different defining criteria. J Clin Hypertens (Greenwich). 2006;8:562–70.

4. Collantes RS, Ong JP, Younossi ZM. The metabolic syndrome and nonalcoholic fatty liver disease. Panminerva Med. 2006;48:41–8.

5. Flegal KM, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999–2000. JAMA. 2002;288:1723–7.

6. Wang Y, Beydoun MA. The obesity epidemic in the United States—gender, age, socioeconomic, racial/ethnic, and geographic characteristics: a systematic review and meta-regression analysis. Epidemiol Rev. 2007;29:6–28.

7. Arner P. Resistin: yet another adipokine tells us that men are not mice. Diabetologia. 2005;48:2203–5.

8. Brambilla G, Cantafora A. Metabolic and cardiovascular disorders in highly inbred lines for intensive pig farming: how animal welfare evaluation could improve the basic knowledge of human obesity. Ann Ist Super Sanita. 2004;40:241–4.

9. Gerrity RG, Natarajan R, Nadler JL, Kimsey T. Diabetes-induced accelerated atherosclerosis in swine. Diabetes. 2001;50:1654–65.

10. Jonsson T, Ahren B, Pacini G, Sundler F, Wierup N, Steen S, Sjoberg T, Ugander M, Frostegard J, et al. A Paleolithic diet confers higher insulin sensitivity, lower C-reactive protein and lower blood pressure than a cereal-based diet in domestic pigs. Nutr Metab (Lond). 2006;3:39.

11. Brisbin IL Jr, Mayer JJ. Problem pigs in a poke: a good pool of data. Science. 2001;294:1280–1.

12. Dyson M, Alloosh M, Vuchetich J, Mokelke E, Sturek M. Components of metabolic syndrome and coronary artery disease in female Ossabaw swine fed excess atherogenic diet. Comp Med. 2006;56:35–45.

13. Dyson MC, Boullion RD, Alloosh M, Mokelke EA, Sturek M. Glucose intolerance and insulin resistance in Ossabaw compared to Yucatan swine [Abstract]. Experimental Biology Meetings, 824.9, 2004.

14. Roytblat L, Rachinsky M, Fisher A, Greemberg L, Shapira Y, Douvdevani A, Gelman S. Raised interleukin-6 levels in obese patients. Obes Res. 2000;8:673–5.

15. Garg R, Tripathy D, Dandona P. Insulin resistance as a proinflammatory state: mechanisms, mediators, and therapeutic interventions. Curr Drug Targets. 2003;4:487–92.

16. Lin Y, Lee H, Berg AH, Lisanti MP, Shapiro L, Scherer PE. The lipopolysaccharide-activated toll-like receptor (TLR)-4 induces synthesis of the closely related receptor TLR-2 in adipocytes. J Biol Chem. 2000; 275:24255–63.

17. Ajuwon KM, Jacobi SK, Kuske JL, Spurlock ME. Interleukin-6 and interleukin-15 are selectively regulated by lipopolysaccharide and interferon-gamma in primary pig adipocytes. Am J Physiol Regul Integr Comp Physiol. 2004;286:R547–53.

18. Webel DM, Finck BN, Baker DH, Johnson RW. Time course of increased plasma cytokines, cortisol, and urea nitrogen in pigs following intraperitoneal injection of lipopolysaccharide. J Anim Sci. 1997;75: 1514–20.

19. Wright KJ, Balaji R, Hill CM, Dritz SS, Knoppel EL, Minton JE. Integrated adrenal, somatotropic, and immune responses of growing pigs to treatment with lipopolysaccharide. J Anim Sci. 2000;78: 1892–9.

20. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest. 2006;116:3015–25.

21. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007;56:1761–72.

22. Gabler NK, Spencer JD, Webel DM, Spurlock ME. n-3 PUFA attenuate lipopolysaccharide-induced down-regulation of toll-like receptor 4 expression in porcine adipose tissue but does not alter the expression of other immune modulators. J Nutr Biochem. 2007 [e-pub ahead of print] Apr 13.

23. Jiang Z, Rothschild MF. Swine genome science comes of age. Int J Biol Sci. 2007;3:129–31.

24. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest. 2003; 112:1821–30.

25. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest. 2003;112:1796–808.

26. Clement K, Viguerie N, Poitou C, Carette C, Pelloux V, Curat CA, Sicard A, Rome S, Benis A, et al. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. FASEB J. 2004; 18:1657–69.

27. Cancello R, Henegar C, Viguerie N, Taleb S, Poitou C, Rouault C, Coupaye M, Pelloux V, Hugol D, et al. Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. Diabetes. 2005;54:2277–86.

28. Sartipy P, Loskutoff DJ. Monocyte chemoattractant protein 1 in obesity and insulin resistance. Proc Natl Acad Sci USA. 2003;100:7265–70.

29. Takahashi K, Mizuarai S, Araki H, Mashiko S, Ishihara A, Kanatani A, Itadani H, Kotani H. Adiposity elevates plasma MCP-1 levels leading to the increased CD11b-positive monocytes in mice. J Biol Chem. 2003; 278:46654–60.

30. Chen A, Mumick S, Zhang C, Lamb J, Dai H, Weingarth D, Mudgett J, Chen H, MacNeil DJ, et al. Diet induction of monocyte chemo-attractant protein-1 and its impact on obesity. Obes Res. 2005;13: 1311–20.

31. Fasshauer M, Paschke R, Stumvoll M. Adiponectin, obesity, and cardiovascular disease. Biochimie. 2004;86:779–84.

32. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA. 2001;286:327–34.

33. Ajuwon KM, Spurlock ME. Palmitate activates the NF-kappaB transcription factor and induces IL-6 and TNFalpha expression in 3T3–L1 adipocytes. J Nutr. 2005;135:1841–6.

34. Inouye KE, Shi H, Howard JK, Daly CH, Lord GM, Rollins BJ, Flier JS. Absence of CC chemokine ligand 2 does not limit obesity-associated infiltration of macrophages into adipose tissue. Diabetes. 2007;56:2242–50.

35. Neels JG, Olefsky JM. Inflamed fat: what starts the fire? J Clin Invest. 2006;116:33–5.

36. Fruebis J, Tsao TS, Javorschi S, Ebbets-Reed D, Erickson MR, Yen FT, Bihain BE, Lodish HF. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci USA. 2001;98: 2005–10.

37. Wu X, Motoshima H, Mahadev K, Stalker TJ, Scalia R, Goldstein BJ. Involvement of AMP-activated protein kinase in glucose uptake stimulated by the globular domain of adiponectin in primary rat adipocytes. Diabetes. 2003;52:1355–63.

38. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun. 1999;257:79–83.

39. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol. 2000;20:1595–9.

40. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab. 2001;86:1930–5.

41. Ogawa Y, Kikuchi T, Nagasaki K, Hiura M, Tanaka Y, Uchiyama M. Usefulness of serum adiponectin level as a diagnostic marker of metabolic syndrome in obese Japanese children. Hypertens Res. 2005; 28:51–7.

42. Matsubara M, Maruoka S, Katayose S. Decreased plasma adiponectin concentrations in women with dyslipidemia. J Clin Endocrinol Metab. 2002;87:2764–9.

43. Hulthe J, Hulten LM, Fagerberg B. Low adipocyte-derived plasma protein adiponectin concentrations are associated with the metabolic syndrome and small dense low-density lipoprotein particles: atherosclerosis and insulin resistance study. Metabolism. 2003;52:1612–4.

44. van der Vleuten GM, van Tits LJ, den Heijer M, Lemmers H, Stalenhoef AF, de Graaf J. Decreased adiponectin levels in familial combined hyperlipidemia patients contribute to the atherogenic lipid profile. J Lipid Res. 2005;46:2398–404.

45. Oana F, Takeda H, Hayakawa K, Matsuzawa A, Akahane S, Isaji M, Akahane M. Physiological difference between obese (fa/fa) Zucker rats and lean Zucker rats concerning adiponectin. Metabolism. 2005;54: 995–1001.

46. Yatagai T, Nagasaka S, Taniguchi A, Fukushima M, Nakamura T, Kuroe A, Nakai Y, Ishibashi S. Hypoadiponectinemia is associated with visceral fat accumulation and insulin resistance in Japanese men with type 2 diabetes mellitus. Metabolism. 2003;52:1274–8.

47. Altomonte J, Harbaran S, Richter A, Dong H. Fat depot-specific expression of adiponectin is impaired in Zucker fatty rats. Metabolism. 2003;52:958–63.

48. Li J, Yu X, Pan W, Unger RH. Gene expression profile of rat adipose tissue at the onset of high-fat-diet obesity. Am J Physiol Endocrinol Metab. 2002;282:E1334–41.

49. Bluher M, Michael MD, Peroni OD, Ueki K, Carter N, Kahn BB, Kahn CR. Adipose tissue selective insulin receptor knockout protects against obesity and obesity-related glucose intolerance. Dev Cell. 2002;3: 25–38.

50. Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R. Hormonal regulation of adiponectin gene expression in 3T3–L1 adipocytes. Biochem Biophys Res Commun. 2002;290:1084–9.

51. Halleux CM, Takahashi M, Delporte ML, Detry R, Funahashi T, Matsuzawa Y, Brichard SM. Secretion of adiponectin and regulation of

apM1 gene expression in human visceral adipose tissue. Biochem Biophys Res Commun. 2001;288:1102–7.

52. Viengchareun S, Zennaro MC, Pascual-Le Tallec L, Lombes M. Brown adipocytes are novel sites of expression and regulation of adiponectin and resistin. FEBS Lett. 2002;532:345–50.

53. Yokota T, Oritani K, Takahashi I, Ishikawa J, Matsuyama A, Ouchi N, Kihara S, Funahashi T, Tenner AJ, et al. Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood. 2000;96:1723–32.

54. Wulster-Radcliffe MC, Ajuwon KM, Wang J, Christian JA, Spurlock ME. Adiponectin differentially regulates cytokines in porcine macrophages. Biochem Biophys Res Commun. 2004;316:924–9.

55. Wulster-Radcliffe MC, Spurlock ME. Adiponectin suppresses proliferation and proinflammatory cytokine production in THP-1 monocytes, and induces caspase 3/7 activity. Adipocytes. 2005;1:35–42.

56. Ajuwon KM, Spurlock ME. Adiponectin inhibits LPS-induced NF-kappaB activation and IL-6 production and increases PPARgamma2 expression in adipocytes. Am J Physiol Regul Integr Comp Physiol. 2005;288:R1220–5.

57. Maeda N, Takahashi M, Funahashi T, Kihara S, Nishizawa H, Kishida K, Nagaretani H, Matsuda M, Komuro R, et al. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes. 2001;50:2094–9.

58. Kappes A, Loffler G. Influences of ionomycin, dibutyryl-cycloAMP and tumour necrosis factor-alpha on intracellular amount and secretion of apM1 in differentiating primary human preadipocytes. Horm Metab Res. 2000;32:548–54.

59. Bruun JM, Lihn AS, Verdich C, Pedersen SB, Toubro S, Astrup A, Richelsen B. Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. Am J Physiol Endocrinol Metab. 2003;285:E527–33.

60. Fasshauer M, Kralisch S, Klier M, Lossner U, Bluher M, Klein J, Paschke R. Adiponectin gene expression and secretion is inhibited by interleukin-6 in 3T3–L1 adipocytes. Biochem Biophys Res Commun. 2003;301:1045–50.

61. Sopasakis VR, Sandqvist M, Gustafson B, Hammarstedt A, Schmelz M, Yang X, Jansson PA, Smith U. High local concentrations and effects on differentiation implicate interleukin-6 as a paracrine regulator. Obes Res. 2004;12:454–60.

62. Hardie DG. Minireview: the AMP-activated protein kinase cascade: the key sensor of cellular energy status. Endocrinology. 2003;144:5179–83.

63. Bruning JC, Michael MD, Winnay JN, Hayashi T, Horsch D, Accili D, Goodyear LJ, Kahn CR. A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. Mol Cell. 1998;2:559–69.

64. Kurth-Kraczek EJ, Hirshman MF, Goodyear LJ, Winder WW. 5′ AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. Diabetes. 1999;48:1667–71.

65. Merrill GF, Kurth EJ, Hardie DG, Winder WW. AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. Am J Physiol. 1997;273:E1107–12.

66. Vavvas D, Apazidis A, Saha AK, Gamble J, Patel A, Kemp BE, Witters LA, Ruderman NB. Contraction-induced changes in acetyl-CoA carboxylase and 5′-AMP-activated kinase in skeletal muscle. J Biol Chem. 1997;272:13255–61.

67. Velasco G, Geelen MJ, Guzman M. Control of hepatic fatty acid oxidation by 5′-AMP-activated protein kinase involves a malonyl-CoA-dependent and a malonyl-CoA-independent mechanism. Arch Biochem Biophys. 1997;337:169–75.

68. Winder WW. Malonyl-CoA–regulator of fatty acid oxidation in muscle during exercise. Exerc Sport Sci Rev. 1998;26:117–32.

69. Le Roy P, Naveau J, Elsen JM, Sellier P. Evidence for a new major gene influencing meat quality in pigs. Genet Res. 1990;55:33–40.

70. Estrade M, Vignon X, Rock E, Monin G. Glycogen hyperaccumulation in white muscle fibres of RN- carrier pigs. A biochemical and ultrastructural study. Comp Biochem Physiol B. 1993;104:321–6.

71. Milan D, Jeon JT, Looft C, Amarger V, Robic A, Thelander M, Rogel-Gaillard C, Paul S, Iannuccelli N, et al. A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. Science. 2000;288:1248–51.

72. Ciobanu D, Bastiaansen J, Malek M, Helm J, Woollard J, Plastow G, Rothschild M. Evidence for new alleles in the protein kinase adenosine monophosphate-activated gamma(3)-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. Genetics. 2001;159:1151–62.

73. Shen W, Sturek M, Liechty EA. Molecular mechanisms for insulin resistance in Ossabaw miniature swine. FASEB J. 2007;21:A1053–c.

74. Lloyd PG, Fang M, Brisbin IL Jr, Andersson L, Sturek M. AMP kinase gene mutation is consistent with a thrifty phenotype (metabolic syndrome) in a population of feral swine. FASEB J. 2006;20:A299.

75. Esfandiari F, You M, Villanueva JA, Wong DH, French SW, Halsted CH. S-Adenosylmethionine attenuates hepatic lipid synthesis in micropigs fed ethanol with a folate-deficient diet. Alcohol Clin Exp Res. 2007;31:1231–9.

76. Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med. 2002;8:1288–95.