

# World Congress on Insulin Resistance, Diabetes, and Cardiovascular Disease

## Part 3

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This is the third of four reports on the 8th Annual World Congress on Insulin Resistance, Diabetes, and Cardiovascular Disease, held on 4–6 November 2010, in Los Angeles, California.

### INSULIN RESISTANCE: LIPID EFFECTS

Ronald Krauss (Berkley, CA) discussed lipid effects of peroxisome proliferator-activated receptors (PPARs). PPAR $\alpha$  activation reduces triglyceride-rich lipoproteins as a consequence of combined intrahepatic actions increasing fatty acid oxidation, increasing apolipoprotein (apo)A-5, and decreasing apoC-3. The LDL phenotype B, defined by small dense LDL cholesterol with low peak particle size, may be considered a specific marker for atherogenic dyslipidemia or, as Krauss termed it, “a fingerprint for this dyslipidemic phenotype” with high triglyceride, low HDL cholesterol, and high levels of apoB for a given LDL cholesterol concentration. A study of lipoprotein particle size and concentration as assessed using nuclear magnetic resonance spectroscopy among 26,836 nondiabetic women in the Women’s Health Study showed that LDL cholesterol was not associated with the pattern B phenotype and that both LDL particle concentration and size were strongly and independently associated with diabetes to nearly the same degree as the A1C level (1). HDL size, HDL cholesterol, triglyceride, and VLDL size also displayed the association with the small dense LDL cholesterol phenotype, suggesting that all these measures reflect the interplay between fatty acids and lipid metabolism as they relate to insulin resistance.

PPAR $\alpha$  activation decreases VLDL cholesterol, changes LDL cholesterol from smaller to larger size, and raises

HDL cholesterol, while the lipoprotein effects of PPAR $\delta$  are less well understood. Both are reported to be activated by hydrolyzed VLDL lipids, although endogenous factors activating these receptors have not been well characterized. In an in vitro study in the absence of albumin, cellular fatty acid uptake determined PPAR $\alpha$  activation but at fatty acid levels not achievable in plasma because of the effect of albumin binding (2). One therefore needs then to look for sources of endogenous PPAR activation by fatty acid; Krauss suggested “that’s where VLDL comes in.” Local VLDL lipolysis may present high concentrations of fatty acid to tissues. Supporting this hypothesis, treatment with P-407, a fatty acid clearance inhibitor, reverses the activation of PPAR $\alpha$  target genes occurring with fasting, implying that endogenous PPAR $\alpha$  activation relies on VLDL triglyceride lipolysis. ApoC3, which inhibits lipoprotein lipase (LPL), can be seen as regulating access to lipoprotein-derived PPAR $\alpha$  ligands. Lipolysis of VLDL-derived fatty acid then can be thought of as an important process for activation of PPAR $\alpha$  both in the liver and in the periphery.

Use of the relatively specific pharmacologic PPAR $\delta$  activator MBX-8025 (Metabolex), in a phase 2 proof-of-concept study of obese individuals with elevated levels of triglyceride and LDL cholesterol and HDL cholesterol <60 mg/dL, decreased triglyceride and fatty acid levels, and increased HDL cholesterol, with a fall in apoB and with reduction in insulin and in homeostasis model assessment of insulin resistance (HOMA-IR)—an effect beyond what one would expect from a typical PPAR $\alpha$  agonist. With use of direct lipoprotein particle measurement by ion

mobility, reduction in LDL cholesterol and increase in HDL cholesterol levels were seen independent of effects on HOMA-IR, with an increase in LDL cholesterol peak diameter, leading to an almost complete conversion of phenotype B to phenotype A associated with reduction in VLDL triglyceride (3). Another PPAR $\delta$  agonist, GW501516, led to 20% reduction in liver fat, which correlated with change in triglyceride levels (4). Krauss speculated that PPAR $\delta$  may complement the effect of PPAR $\alpha$ , reducing peripheral delivery of fatty acid to the liver and perhaps increasing hepatic fatty acid oxidation as is seen with PPAR $\alpha$ . “Time will tell,” he commented, “whether agents such as this will pass muster for clinical efficacy and safety, as well as outcome.”

### INSULIN RESISTANCE: HEPATIC EFFECTS

Marja-Ritta Taskinen (Helsinki, Finland) reviewed aspects of nonalcoholic fatty liver disease (NAFLD), touching on possible mechanisms of its linkage to cardiovascular disease (CVD) and on its association with inflammatory cytokines, fatty acids, hypercoagulation, and atherogenic dyslipidemia—all in the context of insulin resistance (5). The atherogenic lipoprotein triad of large VLDL, small dense LDL cholesterol, and low HDL cholesterol is associated with NAFLD and with increased likelihood of CVD; this is likely the most important risk in individuals with LDL cholesterol at goal. How, she asked, does the liver contribute? There is imbalance between hepatic lipid import of fatty acid derived from adipose tissue, diet, and sugars; Taskinen pointed out that fructose particularly stimulates de novo lipogenesis (6) while decreasing fatty acid oxidation and VLDL assembly and secretion, leading to excess hepatic triglyceride accumulation.

Kinetic studies of the VLDL-intermediate-density lipoprotein-LDL cholesterol cascade reveal a distinct pathway to small dense LDL cholesterol from the larger VLDL1 particles, whereas larger LDL particles are derived from VLDL2 particles (7). In this study, comparing type 2 diabetic with nondiabetic individuals,

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both triglyceride and apoB-100 secretions were markedly increased and secretion of VLDL1 was increased without change in VLDL2 secretion. The VLDL1 triglyceride production rate was positively associated with triglyceride pool size and inversely related to LDL size and to the HDL cholesterol level, implying that in type 2 diabetes the liver produces an excess of VLDL1 particles of similar size and composition to those in nondiabetic individuals, with this overproduction linked to adverse LDL and HDL cholesterol changes. There is a strong direct relationship between VLDL1 triglyceride production and liver fat, which with plasma glucose determines VLDL1 triglyceride production in multivariate analysis (8). Normally, insulin suppresses VLDL1 apoB production, but this does not occur in type 2 diabetes, perhaps in part explaining the accumulation of VLDL particles in insulin resistance. When individuals with low and high liver fat are compared, typical lipid profile differences are seen. VLDL1 triglyceride and apoB-100 production rates were elevated in those with higher liver fat who failed to show the degree of insulin-induced VLDL suppression seen in individuals with low liver fat (9), suggesting dysregulation of the suppressive effects of insulin.

Taskinen discussed a study of obese hyper- versus normotriglyceridemic individuals, both with similar visceral fat but the former with higher liver fat, in association with elevated plasma triglyceride and apoB-100 and reduced HDL cholesterol and LDL peak size. VLDL1 triglyceride and apoB production rates were similarly increased in those with hypertriglyceridemia, and the two measures showed strong correlation. Liver fat content, then, correlates closely with VLDL1 triglyceride and apoB secretion rates and is its major driver. Taskinen observed that hepatic fat, although correlated with plasma triglyceride, only explains a portion of hypertriglyceridemia, with decreased triglyceride clearance being a crucial additional factor, suggesting that “dual mechanisms are required to produce hypertriglyceridemia in obesity.”

Taskinen amplified Krauss's discussion of the critical role of apoC-3 in the metabolism of triglyceride-rich lipoproteins. It is synthesized by the liver and gut and circulates mainly on VLDL and HDL particles. ApoC-3 inhibits LPL, playing a critical role in triglyceride hydrolysis and hence in VLDL cholesterol clearance, with elevated apoC-3 common in insulin resistant states such as obesity

and hypertriglyceridemia (10,11). There is strong negative correlation between apoC-3 and the VLDL cholesterol catabolic rate, whereas hepatic fat moderately correlates with apoC-3. Acute elevation of fatty acid levels increases the apoC-3 production rate in triglyceride-rich lipoproteins (12) and, when central obese men are compared with nonobese men, the former have increased VLDL apoC-3 and VLDL apoB production rates (13).

The pattern, then, is one of dysfunctional adipocytes releasing excess fatty acid and cytokines, enhancing hepatic lipogenesis, and contributing to the imbalance between import and export of triglyceride. Hepatic fat is, independent of visceral fat, the driving force for VLDL cholesterol overproduction in this scenario, with hypertriglyceridemia caused by a dual metabolic defect of overproduction of large VLDL particles due to excess hepatic fat coupled with impaired clearance driven by increased apoC-3. Taskinen wondered whether apoC-3 measurement might be useful in determining cardiometabolic risk.

Sam Klein (St. Louis, MO) further discussed the relationship of liver fat to lipoprotein metabolism, underscoring the powerful effect of weight loss and comparing metabolically normal and abnormal forms of obesity. The major metabolic complications of obesity—insulin resistance, diabetes, dyslipidemia, and NAFLD—all are CVD risk factors. Obesity-related metabolic dysfunction involves fatty acid and adipokines released from both subcutaneous and visceral fat, which, if not oxidized or secreted as VLDL cholesterol, lead to increased hepatic fat. Klein reviewed studies suggesting that the notion of a particularly adverse effect of visceral fat “may not be so true.” Not all obese individuals have metabolic abnormalities. In the National Health and Nutrition Examination Survey study, 30% of obese individuals were metabolically normal. Hepatic fat is, however, strongly associated with metabolic dysfunction, correlating with insulin resistance at the levels of liver, skeletal muscle, and adipose tissue (14). When individuals with hypo- $\beta$ -lipoproteinemia are compared with individuals with NAFLD, with similar intrahepatic triglyceride, the former have greater insulin sensitivity, implying that intrahepatic triglyceride may not itself be the mediator of insulin resistance (15). Lean women have higher VLDL triglyceride production than men at rates similar to those of obese women, and at any BMI level women have more fat than men, but

triglyceride levels remain normal because of higher clearance rates. The VLDL triglyceride secretion in NAFLD reflects triglyceride derived from peripheral fat stores and from de novo lipogenesis rather than from circulating fatty acid per se (16). Klein pointed out the curvilinear relationship between intrahepatic fat and hepatic VLDL triglyceride secretion so that when liver fat exceeds 10% of liver mass there is no further increase in triglyceride secretion, leading to further hepatic triglyceride accumulation. In his studies of individuals with NAFLD, apoB secretion is not increased to as great an extent as VLDL triglyceride, leading to triglyceride enrichment of nascent VLDL particles. Indeed, transgenic mice overexpressing sterol regulatory element binding protein 1A have VLDL particles so large that they appear to have difficulty escaping the endoplasmic reticulum (17).

Weight loss causes reduction in visceral fat and improvement in insulin sensitivity (18–20). Although intrahepatic fat correlates with visceral fat, the two can be separated. When individuals are matched by visceral fat, those with higher liver fat stores exhibit hepatic insulin resistance, with decreased insulin-mediated stimulation of glucose uptake. When matched for liver fat, however, individuals with different visceral fat levels appear metabolically similar (21). VLDL triglyceride secretion is, then, determined by intrahepatic triglyceride rather than by visceral fat. In a study of type 2 diabetic individuals undergoing omentectomy, weight, HOMA-IR, A1C, and requirements for medications failed to change (22), again suggesting that visceral fat “may not be such an important contributor.” Fatty acid kinetics appear to differ between individuals with normal and high intrahepatic triglyceride, with adipocyte levels of the fatty acid transport protein CD36 decreased, but skeletal muscle and hepatic CD36 increased, among those with high liver fat. Obesity may then increase adipocyte fatty acid release while increasing fatty acid uptake by muscle and liver. Caloric restriction decreases intrahepatic triglyceride and improves hepatic insulin sensitivity (18). More marked, but similar, effects are seen following bariatric surgery (23).

Sonia Caprio (New Haven, CT) discussed the importance of hepatic fat in IR in obese adolescents and the potential roles of genetic polymorphisms in pediatric fatty liver (24–28). NAFLD is increasing in prevalence among obese children and is the commonest cause of chronic liver

disease in children, associated with insulin resistance and with abnormalities in glucose and lipid metabolism. In a study of 61 adolescents with BMI Z score 2.2–2.5, 23 had high liver fat and were matched to 20 with similar visceral and intramyocellular fat but lower liver fat (29). The high liver fat group had higher insulin, triglyceride, and alanine transaminase (although within normal range) and lower adiponectin. During a two-step hyperinsulinemic-euglycemic clamp, those with high liver fat manifested less suppression of hepatic glucose production and lipolysis at a lower insulin dose and skeletal muscle resistance to high dose insulin infusion, as well as defects in  $\beta$ -cell function. The patatin-like phospholipase 3 gene is expressed in liver and adipose tissue. The rs738409 variant is associated with increased hepatic fat in adults (30). Caprio's group studied 85 obese children and adolescents with magnetic resonance imaging, glucose tolerance testing, genotyping, and insulin clamp studies. Homozygotes for the G polymorphism had increased hepatic fat, although they found no difference in metabolic characteristics. Other groups have confirmed an association of GG with liver fat (31), with evidence that it predicts steatohepatitis and even fibrosis on biopsy (32) without effect on insulin resistance. The rs780094 and rs1260326 SNPs in the glucokinase regulatory protein (*GCKR*) gene have been found to be associated with triglyceride levels (33–35). In Caprio's studies, those with the TT allele have high serum triglyceride and increased hepatic fat. The *apoC-3* gene polymorphisms C-482T (rs2854116) and T-455C (rs2854117) have been reported to be associated with NAFLD (11), although Caprio has not confirmed effects on triglyceride or hepatic fat.

Arun Sanyal (Richmond, VA) discussed current approaches to treatment of NAFLD. Modifiable risk factors are weight gain, insulin resistance, and diabetes, and nonmodifiable are age, race, genetic background, and baseline histology. The NAFLD activity score, which combines measures of steatosis, inflammation, and fibrosis, can give additional information as to candidates for treatment. Given the strong associations with diabetes and CVD, one could argue that all individuals with NAFLD and, particularly, all with nonalcoholic steatohepatitis (NASH) should be treated. If focus is on prevention of liver disease, however, one would treat patients with NASH having a high activity score, risk factors

for progression, or increasing degrees of fibrosis. At present, Sanyal said, we do not have sufficient information to make recommendations on approaches to reducing hepatic cancer development. The goals of liver disease treatment are to reduce liver-related mortality; induce disease regression; reverse steatohepatitis; improve the severity of steatosis, ballooning, inflammation, and fibrosis; and slow progression. The ideal treatment would be rational, easily available, highly effective, and safe. Sanyal reviewed the current hypothesis of pathogenesis, that IR leads to elevations in levels of fatty acids, insulin, and cytokines, with steatosis and metabolic dysregulation causing hepatic oxidative stress, mitochondrial injury, and what has been more recently recognized of endothelial reticulum damage and endoplasmic reticulum stress, leading to inflammatory signaling, apoptosis, and cell death, with subsequent stellate cell activation leading to fibrosis. Pilot studies have been promising with antioxidants and thiazolidinediones. In patients with NASH randomized to 800 units daily of vitamin E, 30 mg pioglitazone daily, or placebo, liver biopsy at baseline and after 96 weeks was performed to analyze the effect on NAFLD activity score, with particular attention to cytologic ballooning, a major marker of activity, and lack of worsening of fibrosis, as ballooning may resolve as the disease progresses to cirrhosis (36). Blinded review of baseline and final biopsies at central location showed 18% of vitamin E, 17% of placebo, and 27% of pioglitazone cases were found not to have ballooning at baseline, a baseline inequality which led pioglitazone to appear to be less effective. Both agents improved steatosis, lobular inflammation, and the severity of ballooning and increased the proportion of subjects whose ballooning decreased, and both increased the proportion of patients with resolution of NASH. Pioglitazone was associated with somewhat lower rates of worsening of fibrosis, and both agents showed a trend to improved fibrosis score. Body weight was stable with placebo and vitamin E but showed progressive increase with pioglitazone, although only with this agent did HOMA-IR improve. Although there have been questions as to the safety of vitamin E, Sanyal suggested that this may reflect concomitant administration of high doses of zinc, which can deplete copper, increasing liver abnormality. In a study recently presented at the American Association for the Study of Liver Diseases meeting, metformin was compared with

vitamin E and with placebo for NAFLD in 173 children, with metformin having no benefit. Although vitamin E improved resolution of steatohepatitis and ballooning, it did not improve the overall fibrosis score, and liver function improvement was not sustained after the first year. Intracellular oxidative stress depletes glutathione, activating  $\gamma$ -glutamyl transphosphatase, which breaks down extracellular  $\gamma$ -glutamylated amino acids. Vitamin E responders consistently showed reduction in these amino acids. Vitamin E decreases sterol regulatory element-binding protein-1c. Carnitine acyl transferase may be involved, as the vitamin E response is seen with high carnitine-to-acyl-carnitine ratios, suggesting linkage to the degree of mitochondrial dysfunction. Vitamin E also decreases sphingosine levels in responders and so appears to act both on oxidative stress and on glutathione turnover. It is interesting that vitamin E is also being studied for macular degeneration and for prostate cancer prevention, so we may see a resurgence of interest in this compound. Another important area to be explored will involve combinations of therapies.

#### **INSULIN RESISTANCE: ADIPOCYTE EFFECTS**

Sam Cushman (Bethesda, MD) discussed multisizer Coulter counter analyses of adipocyte cell size in rosiglitazone-treated rats, showing an increase in the "peak" of small cells appearing to be adipocytes, with multiple measurements over a 134-day period appearing to show a 55-day cycle of small cells developing into larger cells with subsequent recruitment of additional small cells "which will then fill up." "The growth," he said, "is not simply one of small cells becoming larger cells." Thiazolidinedione treatment markedly increases the ratio of small cells to large cells, suggesting elimination of a physiologic feedback loop. Comparing C57Bl/6 (obesity-prone) with FVB/N (obesity-resistant) rat strains showed that on a high-fat diet there is ongoing recruitment of small cells, with evidence that change in adipose tissue mass ultimately involves apoptosis of large cells and recruitment of small cells to increase the capacity to absorb energy flux. In attempts to extend this to humans comparing 11 insulin resistant with 15 equally obese insulin-sensitive individuals, again smaller and larger cell populations were seen, with the small-to-large cell ratio higher in the insulin resistant group. Insulin resistant individuals may recruit relatively

dysfunctional small cells, which could then lead to lack of feedback inhibition of further recruitment of small adipocytes.

Phillip Scherer (Dallas, TX) stated, "We've made the transition of adipose tissue from flubber to endocrine tissue." There are hundreds of cytokines produced by adipose tissue involved in energy homeostasis, including adiponectin and leptin, playing roles in inflammation and influencing liver, brain, the  $\beta$ -cell, reproductive tissues, muscle, and the vasculature and having effect on tumor cells. A crucial question is why  $\beta$ -cells fail in the setting of insulin resistance, which is related to the larger issue of ectopic lipid in liver, muscle, and  $\beta$ -cells, which in turn is related to failure to adequately expand subcutaneous adipose tissue for storage of nutrient excess (37).

Adipocyte hypoxia may be an issue. The relative adipose tissue vascular density is reduced in *ob/ob* mice, with evidence of areas of ischemia. In a study with selective adipose tissue overexpression of vascular endothelial growth factor in an obesity model, glucose tolerance improved. Thus, adipose tissue expansion may engender hypoxia. Normally, hypoxia increases adipocyte hypoxia-inducible factor  $1\alpha$  expression, which should increase its vascular supply, but in obesity adipose tissue fibrosis occurs, causing necrosis and subsequent inflammation. Alternatively, enlarging adipocytes may inherently induce proinflammatory cytokine expression, causing inflammation. One role of macrophages is to phagocytose lipid from necrotic fat cells, causing foam cell-like macrophage changes, one of the main mediators of insulin resistance, with agents that prevent macrophage infiltration improving insulin sensitivity. Adipocytes secrete chemokines and acute-phase reactants, such as a nonhepatic form of serum amyloid A3 (38). Interestingly, fasting induces macrophage infiltration and increases adipose tissue vascular permeability in a physiologic and fully reversible fashion (39).

Adiponectin levels may be seen as an integrated measure of adipose tissue health and what is termed "metabolic flexibility": the readiness to adapt to either feeding or fasting conditions (40). Mice overexpressing adiponectin can gain more weight with less liver fat, display improved  $\beta$ 3-adrenergic stimulation of insulin secretion, and have higher levels of adipogenic precursor cells. With high circulating fatty acid levels, they can be oxidized, can be stored as triglyceride,

or can be used to increase ceramide synthesis (41). Plasma ceramides are increased in obese type 2 diabetes (42), and insulin resistance is associated with increased intramyocellular lipid concomitant with elevations in ceramide (43). Thus, ceramide may play an important role in the pathway from healthy to dysfunctional adipose tissue, the latter promoting macrophage activation with subsequent lipotoxicity effects in other tissues (44).

Edgar Engleman (Stanford, CA) discussed the adaptive immune response in adipose tissue and its relationship to insulin resistance, noting that most but not all T cells that recognize self-antigens are removed during T-cell development in the thymus. Autoreactive CD4 and CD8 T cells are held in check by T-regulatory (Treg) cells and other regulatory mechanisms, while autoimmunity results when these mechanisms fail, with autoimmune disease occurring when autoreactive T cells or antibodies attack or destroy tissue. Type 1 diabetes is a classic autoimmune disease in this regard, and the autoimmune process can be prevented or aborted by immunotherapy. It is possible that type 2 diabetes has autoimmune aspects, with contribution by the inflammatory macrophages that accumulate in adipose tissue; proinflammatory T cells also infiltrate these tissues and may play an important pathogenic role in disease. Engleman discussed the possible role of T cells in control of body weight and glucose homeostasis. The  $T_H1$  subset of  $CD4^+$  T lymphocytes secretes interferon- $\gamma$  and tumor necrosis factor- $\alpha$ , promoting cellular immunity and inflammation, while  $T_H2$  cells secrete interleukins-4, -5, and -13, promoting humoral immunity, Treg (Foxp3 $^+$ ) cells secrete interleukin-10 and transforming growth factor- $\beta$ , suppressing the immune response. With a high-fat diet, interferon- $\gamma$ -secreting  $T_H1$  cell increase in rodent visceral and subcutaneous fat, with concomitant decrease in visceral fat  $T_H2$  cells and a profound reduction in Tregs. Only certain T-cell receptors are present in visceral fat, which may react to a specific antigen or set of antigens. Rag-null mice lacking T cells gain more weight and show worse glucose tolerance and insulin resistance on a high-fat diet so that the net effect of T cells may not always promote worsening of glucose homeostasis. In this model, adoptive transfer of CD4 but not CD8 T cells normalizes weight gain (45). Administration of anti-CD3 antibody led to recovery of Treg cells in visceral fat after

9 weeks, to normalization of glucose tolerance, and to change in macrophage types in adipose tissue from the proinflammatory M1 to anti-inflammatory M2 interleukin-secreting macrophages. Obesity predisposes to increased ratio of  $T_H1$ -to-Treg cells in human visceral adipose tissue. The role of T cells in obesity and type 2 diabetes has been particularly studied in rodent models, with Treg and  $T_H2$  cells predominating in adipose tissue in lean mice, secreting interleukin-10 and preventing inflammation. In obese mice, there is a shift to  $CD8^+$  and  $T_H1$  cells, which proceeds influx of inflammatory macrophages and which appears to be involved in the regulation of weight gain. The T-cell response may be antigen driven, but the mediating antigen is not known. These T-cell-mediated effects are reversible during disease development in this model (45).

Jorge Plutzky (Boston, MA) discussed the integration of energy balance with vascular biology, noting that in metabolism the notion of energy balance addresses the handling of fatty acids and glucose, with systems having evolved to address storage, combustion, and survival, driven by issues of increasing adiposity in the existing environment. Fatty acids pack energy considerably more tightly than glucose and glycogen, requiring minimal water for storage. Fatty acids are structurally diverse and are transported in a highly complex system. Prandial measures may be particularly strongly related to cardiovascular risk both for nonfasting triglyceride (46) and for glucose (47). The PPAR-retinoid X receptor (RXR) complex "sits in the middle of this balance," Plutzky said, involved both in glucose and fatty acid metabolism and playing roles in obesity, inflammation, and atherosclerosis. Because the nuclear receptor is an obligate heterodimer with RXR, we should expand our study to assess the roles of retinoids in understanding the system (48) and it appears particularly noteworthy that PPAR-RXR ligands are nutrient derived. The interactions of different lipoproteins with different lipases, of VLDL with LPL, and of HDL with endothelial lipase (EL), suggest a high degree of selectivity and specificity. The combination of LPL with VLDL particularly activates PPAR $\alpha$ , with lesser effect on PPAR $\delta$  and still less on PPAR $\gamma$ . Another aspect of this system pertains to EL, identified in 1999, acting to a greater extent on phospholipid than on triglyceride, with particular effect on

HDL cholesterol (49). HDL cholesterol can inhibit adhesion molecules, and the association of HDL cholesterol with EL appears to lead to release of activator(s) of PPAR $\alpha$ , which may initiate this process (50). EL loss-of-function variants are associated with high HDL cholesterol but fail to offer protection against CVD (51)—a caveat in the development of HDL cholesterol—increasing drugs, which may, like torcetrapib, have adverse effect rather than benefit.

The role of RXR is often overlooked. Its ligand is derived from ingestion of  $\beta$ -carotene or retinyl esters through the action of alcohol dehydrogenase, leading to formation of retinaldehyde, a precursor for retinoic acid, which in turn is metabolized to 9-*cis* retinoic acid. Although this is a ligand for RXR, it is not clear that it plays a physiologic role. Retinoic acid also forms all-*trans* retinoic acid, a ligand for the retinoic acid receptor, which heterodimerizes with RXR, with the retinoic acid receptor–RXR heterodimer inhibiting RXR–PPAR. Mice not expressing retinaldehyde dehydrogenase 1 fail to generate retinoic acid, with reduction in diet-induced obesity by increasing body temperature and otherwise increasing energy dissipation, and also show defects in gluconeogenesis, with compensatory increase in fatty acid oxidation (52). Retinaldehyde may, then, be an important mediator of glucose metabolism.

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## References

- Mora S, Otvos JD, Rosenson RS, Pradhan A, Buring JE, Ridker PM. Lipoprotein particle size and concentration by nuclear magnetic resonance and incident type 2 diabetes in women. *Diabetes* 2010;59:1153–1160
- Ruby MA, Goldenson B, Orasanu G, Johnston TP, Plutzky J, Krauss RM. VLDL hydrolysis by LPL activates PPAR- $\alpha$  through generation of unbound fatty acids. *J Lipid Res* 2010;51:2275–2281
- Caulfield MP, Li S, Lee G, et al. Direct determination of lipoprotein particle sizes and concentrations by ion mobility analysis. *Clin Chem* 2008;54:1307–1316
- Risérus U, Sprecher D, Johnson T, et al. Activation of peroxisome proliferator-activated receptor (PPAR) $\delta$  promotes reversal of multiple metabolic abnormalities, reduces oxidative stress, and increases fatty acid oxidation in moderately obese men. *Diabetes* 2008;57:332–339
- Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with non-alcoholic fatty liver disease. *N Engl J Med* 2010;363:1341–1350
- Stanhope KL, Schwarz JM, Keim NL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest* 2009;119:1322–1334
- Adiels M, Borén J, Caslake MJ, et al. Overproduction of VLDL1 driven by hyperglycemia is a dominant feature of diabetic dyslipidemia. *Arterioscler Thromb Vasc Biol* 2005;25:1697–1703
- Adiels M, Taskinen MR, Packard C, et al. Overproduction of large VLDL particles is driven by increased liver fat content in man. *Diabetologia* 2006;49:755–765
- Adiels M, Westerbacka J, Soro-Paavonen A, et al. Acute suppression of VLDL1 secretion rate by insulin is associated with hepatic fat content and insulin resistance. *Diabetologia* 2007;50:2356–2365
- Sacks FM, Alaupovic P, Moye LA, et al. VLDL, apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) trial. *Circulation* 2000;102:1886–1892
- Petersen KF, Dufour S, Hariri A, et al. Apolipoprotein C3 gene variants in non-alcoholic fatty liver disease. *N Engl J Med* 2010;362:1082–1089
- Pavlic M, Valéro R, Duez H, et al. Triglyceride-rich lipoprotein-associated apolipoprotein C-III production is stimulated by plasma free fatty acids in humans. *Arterioscler Thromb Vasc Biol* 2008;28:1660–1665
- Chan DC, Nguyen MN, Watts GF, Barrett PH. Plasma apolipoprotein C-III transport in centrally obese men: associations with very low-density lipoprotein apolipoprotein B and high-density lipoprotein apolipoprotein A-I metabolism. *J Clin Endocrinol Metab* 2008;93:557–564
- Korenblat KM, Fabbrini E, Mohammed BS, Klein S. Liver, muscle, and adipose tissue insulin action is directly related to intrahepatic triglyceride content in obese subjects. *Gastroenterology* 2008;134:1369–1375
- Amaro A, Fabbrini E, Kars M, et al. Dissociation between intrahepatic triglyceride content and insulin resistance in familial hypobetalipoproteinemia. *Gastroenterology* 2010;139:149–153
- Fabbrini E, Mohammed BS, Magkos F, Korenblat KM, Patterson BW, Klein S. Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. *Gastroenterology* 2008;134:424–431
- Horton JD, Shimano H, Hamilton RL, Brown MS, Goldstein JL. Disruption of LDL receptor gene in transgenic SREBP-1a mice unmasks hyperlipidemia resulting from production of lipid-rich VLDL. *J Clin Invest* 1999;103:1067–1076
- Kirk E, Reeds DN, Finck BN, Mayurranjan SM, Patterson BW, Klein S. Dietary fat and carbohydrates differentially alter insulin sensitivity during caloric restriction. *Gastroenterology* 2009;136:1552–1560
- Tiikkainen M, Bergholm R, Rissanen A, et al. Effects of equal weight loss with orlistat and placebo on body fat and serum fatty acid composition and insulin resistance in obese women. *Am J Clin Nutr* 2004;79:22–30
- Ross R, Dagnone D, Jones PJ, et al. Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. A randomized, controlled trial. *Ann Intern Med* 2000;133:92–103
- Fabbrini E, Magkos F, Mohammed BS, et al. Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proc Natl Acad Sci USA* 2009;106:15430–15435
- Fabbrini E, Tamboli RA, Magkos F, et al. Surgical removal of omental fat does not improve insulin sensitivity and cardiovascular risk factors in obese adults. *Gastroenterology* 2010;139:448–455
- Gregor MF, Yang L, Fabbrini E, et al. Endoplasmic reticulum stress is reduced in tissues of obese subjects after weight loss. *Diabetes* 2009;58:693–700
- Shneider BL, González-Peralta R, Roberts EA. Controversies in the management of pediatric liver disease: hepatitis B, C and NAFLD: summary of a single topic conference. *Hepatology* 2006;44:1344–1354
- Loomba R, Sirlin CB, Schwimmer JB, Lavine JE. Advances in pediatric nonalcoholic fatty liver disease. *Hepatology* 2009;50:1282–1293
- Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C. Prevalence of fatty liver in children and adolescents. *Pediatrics* 2006;118:1388–1393
- Cali AM, De Oliveira AM, Kim H, et al. Glucose dysregulation and hepatic steatosis in obese adolescents: is there a link? *Hepatology* 2009;49:1896–1903
- Fabbrini E, deHaseth D, Deivanayagam S, Mohammed BS, Vitola BE, Klein S. Alterations in fatty acid kinetics in obese adolescents with increased intrahepatic triglyceride content. *Obesity (Silver Spring)* 2009;17:25–29
- D'Adamo E, Cali AM, Weiss R, et al. Central role of fatty liver in the pathogenesis of insulin resistance in obese adolescents. *Diabetes Care* 2010;33:1817–1822

30. Santoro N, Kursawe R, D'Adamo E, et al. A common variant in the patatin-like phospholipase 3 gene (PNPLA3) is associated with fatty liver disease in obese children and adolescents. *Hepatology* 2010; 52:1281–1290
31. Goran MI, Walker R, Le KA, et al. Effects of PNPLA3 on liver fat and metabolic profile in Hispanic children and adolescents. *Diabetes* 2010;59:3127–3130
32. Valenti L, Al-Serri A, Daly AK, et al. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2010;51:1209–1217
33. Tam CH, Ma RC, So WY, et al. Interaction effect of genetic polymorphisms in glucokinase (GCK) and glucokinase regulatory protein (GCKR) on metabolic traits in healthy Chinese adults and adolescents. *Diabetes* 2009;58:765–769
34. Vaxillaire M, Cavalcanti-Proença C, Dechaume A, et al.; DESIR Study Group. The common P446L polymorphism in GCKR inversely modulates fasting glucose and triglyceride levels and reduces type 2 diabetes risk in the DESIR prospective general French population. *Diabetes* 2008; 57:2253–2257
35. Beer NL, Tribble ND, McCulloch LJ, et al. The P446L variant in GCKR associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. *Hum Mol Genet* 2009;18:4081–4088
36. Sanyal AJ, Chalasani N, Kowdley KV, et al.; NASH CRN. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *N Engl J Med* 2010;362:1675–1685
37. Unger RH, Scherer PE. Gluttony, sloth and the metabolic syndrome: a roadmap to lipotoxicity. *Trends Endocrinol Metab* 2010;21:345–352
38. Lin Y, Rajala MW, Berger JP, Moller DE, Barzilai N, Scherer PE. Hyperglycemia-induced production of acute phase reactants in adipose tissue. *J Biol Chem* 2001; 276:42077–42083
39. Kosteli A, Sugaru E, Haemmerle G, et al. Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. *J Clin Invest* 2010;120:3466–3479
40. Asterholm IW, Scherer PE. Enhanced metabolic flexibility associated with elevated adiponectin levels. *Am J Pathol* 2010;176:1364–1376
41. Holland WL, Knotts TA, Chavez JA, Wang LP, Hoehn KL, Summers SA. Lipid mediators of insulin resistance. *Nutr Rev* 2007;65:S39–S46
42. Haus JM, Kashyap SR, Kasumov T, et al. Plasma ceramides are elevated in obese subjects with type 2 diabetes and correlate with the severity of insulin resistance. *Diabetes* 2009;58:337–343
43. Coen PM, Dubé JJ, Amati F, et al. Insulin resistance is associated with higher intramyocellular triglycerides in type I but not type II myocytes concomitant with higher ceramide content. *Diabetes* 2010;59:80–88
44. Cusi K. The role of adipose tissue and lipotoxicity in the pathogenesis of type 2 diabetes. *Curr Diab Rep* 2010;10:306–315
45. Winer S, Chan Y, Paltser G, et al. Normalization of obesity-associated insulin resistance through immunotherapy. *Nat Med* 2009; 15:921–929
46. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *JAMA* 2007;298:299–308
47. DECODE Study Group, European Diabetes Epidemiology Group. Is the current definition for diabetes relevant to mortality risk from all causes and cardiovascular and noncardiovascular diseases? *Diabetes Care* 2003;26:688–696
48. Brown JD, Plutzky J. Peroxisome proliferator-activated receptors as transcriptional nodal points and therapeutic targets. *Circulation* 2007;115:518–533
49. Cockerill GW, Rye KA, Gamble JR, Vadas MA, Barter PJ. High-density lipoproteins inhibit cytokine-induced expression of endothelial cell adhesion molecules. *Arterioscler Thromb Vasc Biol* 1995;15:1987–1994
50. Ahmed W, Orasanu G, Nehra V, et al. High-density lipoprotein hydrolysis by endothelial lipase activates PPARalpha: a candidate mechanism for high-density lipoprotein-mediated repression of leukocyte adhesion. *Circ Res* 2006;98:490–498
51. Vergeer M, Cohn DM, Boekholdt SM, et al. Lack of association between common genetic variation in endothelial lipase (LIPG) and the risk for CAD and DVT. *Atherosclerosis* 2010;211:558–564
52. Ziouzenkova O, Orasanu G, Sharlach M, et al. Retinaldehyde represses adipogenesis and diet-induced obesity. *Nat Med* 2007;13:695–702