Perspectives in Diabetes

Transcriptional Control of Apolipoprotein A-I Gene Expression in Diabetes

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Cardiovascular disease continues to be the leading cause of mortality in diabetes. One of the factors contributing to the increased risk is the high prevalence rate of low plasma concentrations of HDL cholesterol. Multiple potential mechanisms account for the cardioprotective effects of HDL and its main protein apolipoprotein (apo) A-I. The reduced plasma concentrations of HDL could be the result of increased fractional clearance of HDL and reduced expression of apo A-I. In animal models of diabetes and in cell cultures treated with high concentrations of glucose, apo A-I expression is reduced. In this review we will discuss the alterations in transcriptional control of apo A-I in diabetes. The role of select nutritional and hormonal alterations commonly found in diabetes will be reviewed. Specifically, we will review the literature on the effect of hyperglycemia, hypoinsulinemia, and ketoacidosis, as well as the role of various mediators of insulin resistance, such as fatty acids, cytokines, and prostanoids, on apo A-I promoter activity. Identifying the mechanisms that modulate apo A-I gene expression will aid in the new development of therapeutic agents that increase plasma apo A-I and HDL concentrations. *Diabetes* 53:513–520, 2004

Cardiovascular disease (CVD) continues to be the leading cause of morbidity and mortality in subjects with diabetes (1). Diabetic subjects have a two- to fourfold increase in the risk of CVD. The 7-year mortality in diabetic subjects without history of prior myocardial infarction is equal to the risk in nondiabetic subjects with established history of myocardial infarction (2). These observations were the bases for designating diabetes as a coronary heart disease (CHD) equivalent. It is disconcerting that at a time when the risk of cardiovascular morality is decreasing in the general population, diabetic people are experiencing no significant decrease in risk, and in diabetic women there is a disturbing trend of increasing cardiovascular mortality (1). These statistics suggest that the currently available tools used in managing cardiovascular disease are not equally effective in diabetic subjects. There are multiple reasons for the discrepancy between the diabetic subjects and the general population. One potential reason is the difference in the plasma lipid profile. A notable difference in the plasma lipid composition between diabetic and nondiabetic subjects is the increased prevalence of hypertriglyceridemia and low HDL cholesterol levels (3). However, it is noteworthy that subjects with type 1 diabetes, unlike those with type 2 diabetes, do not usually have low HDL cholesterol levels (3). It is possible that one of the reasons cardiovascular risk in diabetic subjects has not been decreasing at the same rate as in nondiabetic subjects is that the most prevalent approach to the management of dyslipidemias with statins targets LDL cholesterol levels while providing only modest effects on triglyceride and HDL cholesterol concentrations. It is tempting to speculate that when the problem of hypertriglyceridemia and low HDL cholesterol is more frequently managed, the incidence of cardiovascular disease in diabetes may be reduced.

The relative role of hypertriglyceridemia and low HDL cholesterol in diabetes-related accelerated atherosclerosis is not known. In the U.K. Prospective Diabetes Study (UKPDS), HDL cholesterol was one of the best predictors of cardiovascular events second only to LDL cholesterol (4). Epidemiological studies as well as studies in animal models of atherosclerosis support the cardioprotective role of HDL (5). In addition, interventional trials, notably the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT), lends direct evidence in favor of therapeutic targeting of HDL (6).

The major lipoprotein constituents of HDL are apolipoprotein (apo) A-I and A-II. Whereas apo A-I has cardioprotective properties, apo A-II has been associated with increased risk of atherosclerosis in animal models (5). In this article, we will briefly review the evidence in favor of the cardioprotective properties of apoA-I and discuss the potential mechanisms for reduced apo A-I expression in diabetes.

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apo, apolipoprotein; CHD, coronary heart disease; COX, cyclooxygenase; CVD, cardiovascular disease; FFA, free fatty acid; HNF, hepatocyte nuclear factor; IL, interleukin; IRCE, insulin response core element; nTRE, negative thyroid hormone response element; pH-RE, pH-responsive element; PKC, protein kinase C; PMA, phorbol myristate acetate; TNF-α, tumor necrosis factor-α; UKPDS, U.K. Prospective Diabetes Study; VA-HIT, Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial.

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TABLE 1
Antiatherogenicity of apo A-I: clinical and experimental evidence

- Epidemiological studies: Inverse association between apo A-I levels and CHD outcome
- Familial deficiency in apo A-I associated with premature CHD
  - Mutations in AI/CIII/AIV gene
  - Tangier disease
  - Familial hypoalphalipoproteinemia ± hypertriglyceridemia
- Human apo A-I is protective in transgenic animal models and in somatic gene transfer experiments:
  - Cholesterol-fed C57BL/6 mice.
  - Apo-E knockout mice
  - Human apo (a) transgenic mice
  - Cholesterol-fed rabbits
  - Human apo A-I transgenic rabbits
  - LDL receptor–deficient mice
- Apo A-I infusion in cholesterol-fed rabbits protects against CHD
- Treatment with apo A-I Milano reduces atherosclerosis in:
  - Apo E–deficient mice
  - Hypercholesterolemic rabbits following vascular injury

For review, see refs. 5, 8, and 9.

CARDIOPROTECTIVE ROLE OF APO A-I

The inverse relationship between HDL cholesterol and CHD has been described in several large epidemiological studies (5). In the Framingham study, the protective effect of HDL cholesterol was so remarkable that when the concentrations reached 85 mg/dl or at least 65 mg/dl, the elevated concentrations of LDL cholesterol did not appear to increase CHD risk (7). Whereas every 1% reduction in LDL cholesterol reduces CHD risk by 2%, raising HDL cholesterol level the same amount reduces CHD risk by 3% (7). Although not unequivocally established in clinical trials, it appears that raising HDL is more effective than reducing LDL cholesterol. In support of this notion is the observation that pharmacologic interventions with gemfibrozil in subjects with low HDL cholesterol resulted in a 22% reduction in CHD risk that was attributed to the 7.2% increase in HDL cholesterol level (6). Review of the genetic disorders associated with low HDL cholesterol suggests that when reduced apo A-I expression is the underlying mechanism of low HDL, the risk of CHD is increased.

In addition to the cardioprotective role, HDL cholesterol may have a role in preventing strokes and Alzheimer's disease and may ameliorate age-related changes in blood-brain barrier integrity (5). However, the evidence in favor of this neuroprotective role of HDL is not as conclusive as the data supporting its cardioprotective properties.

The cardioprotective effect of HDL has been attributed mostly, but not exclusively, to its major protein constituent, namely apo A-I (5,8–10). Some epidemiological studies confirm the inverse correlation between serum apo A-I concentrations and cardiovascular events (5). More importantly, various experimental manipulations targeting increased production of apo A-I are associated with reduced atherogenicity (Table 1).

The potential mechanisms of the cardioprotective effects of apo A-I include enhancement of reverse cholesterol transport, attenuation of oxidative stress, increased peroxonase activity, and enhanced anticoagulant activity (5). Despite the uncertainties as to the mechanisms of cardioprotection, the overall evidence supporting an antiatherogenic role of apo A-I is convincing. Therefore, elucidation of the biochemical pathways regulating apo A-I gene expression is fundamental to the development of new therapies for preventing atherosclerosis.

In type 2 diabetes, the reduced serum HDL cholesterol levels have been attributed to increased fractional clearance of HDL (11). This has been attributed to variety of changes including depletion of HDL of its cholesterol ester content. Thus, increased VLDL production, at least partly because of increased fatty acid flux to the liver, promotes exchange of triglyceride for HDL cholesterol ester through the action of cholesterol ester transfer protein. The triglyceride-enriched HDL is then hydrolyzed through the action of hepatic lipase or lipoprotein lipase. Apo A-I dissociates from smaller HDL and is filtered by the glomerulus and degraded in renal tubular cells (11).

In this article, we will focus on the effects of diabetes-related changes in the metabolic milieu on the transcriptional control of the apo A-I gene.

TRANSCRIPTIONAL REGULATION OF APO A-I GENE

Expression of the apo A-I gene is regulated primarily at the transcriptional level. The apo A-I gene promoter contains a TATA-like motif close to the transcriptional start site, while further 5′, several *cis* elements regulate expression of the gene in either a positive or negative manner in response to changes in the hormonal or metabolic status. For example, several hormones have been shown to stimulate transcription of the apo A-I gene. Thyroid hormones, retinoids, estrogens, and glucocorticoids have been shown to induce apo A-I promoter activity and gene expression through proximal promoter elements located between nucleotides −235 and −144 (relative to the transcriptional start site, +1) (12). While thyroid hormones as well as retinoids act on the apo A-I promoter directly through their nuclear receptors, no glucocorticoid or estrogen receptor binding sites are present within this region. Instead, glucocorticoids stimulate binding of the transcriptional activator hepatocyte nuclear factor (HNF)-3β to the promoter (13), leading to an increase in transcription. Likewise, estradiol promotes the interaction between HNF-3β and the orphan nuclear receptor HNF-4 on the apo A-I promoter, most likely due to inactivation of the estradiol-responsive transcriptional corepressor RIP-140, increasing apo A-I gene transcription (14). Furthermore, several transcriptional repressors have been shown to suppress apo A-I gene transcription, including ARP-1 (apo A-I repressor protein-1), HNF-4, and thyroid hormone receptor. However, binding of a thyroid hormone receptor monomer to a negative thyroid hormone response element (nTRE) located 3′ of the apo A-I gene TATA-box may suppress apo A-I gene transcription only when removed from the full-length promoter (15). Further 5′ from the transcriptional start site, an insulin response core element (IRCE) is located between nucleotides −404 and −411 (16). This element binds to the ubiquitous transcription factor Sp1 and is responsible for the induction of the apo A-I gene by insulin. Thus, expression of the apo A-I gene is subject to regulation by several hormone and metabolic signaling pathways, many of which are altered in diabetes.

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EFFECT OF GLUCOSE AND INSULIN ON APO A-I GENE EXPRESSION

Hepatic expression of apo A-I protein and its mRNA is reduced in streptozotocin-induced diabetic rats (16). Apo A-I gene expression in insulin-resistant diabetic animal models is not well studied. To further study the mechanisms by which glucose and insulin regulate the levels of apo A-I, we have used the human hepatoma cell line HepG2. This cell line retains the ability to synthesize and secrete apo A-I as well as other proteins produced by normal hepatocytes. In addition, these cells express insulin receptor and glucose transporter proteins and therefore are a suitable model to study the effects of glucose and insulin on apo A-I expression.

Treatment of HepG2 cells with 22.4 mmol/l dextrose for 48 h results in a 50% reduction in apo A-I mRNA levels, whereas treatment with 100 μU/ml of insulin causes a twofold increase in apo A-I mRNA levels relative to control cells maintained in media containing 5.5 mmol/l dextrose (16). Treatment of the cells with both high concentrations of dextrose and insulin was associated with 1.3-fold induction of apo A-I mRNA, which is an intermediate response to that of dextrose and insulin. The transcriptional activity of apo A-I promoter is also suppressed by dextrose and stimulated by insulin in a dose-dependent fashion (16). Deletional analysis of the promoter showed that a 50-bp fragment spanning nucleotides −425 to −376 mediates the effects of both dextrose and insulin. Within this DNA fragment, between −411 and −404, an IRCE was identified. Mutation of this motif abolishes the effects of both insulin and dextrose. Thus, it appears that a single *cis*-acting element within the promoter regulates the effects of both insulin and dextrose on transcriptional activity of apo A-I gene. However, the fact that insulin but not dextrose alters IRCE binding activity suggests that there are additional carbohydrate-responsive elements remaining to be characterized, and that the IRCE may have only a permissive role for the inhibitory effects of dextrose (16).

The stimulatory effect of insulin on apo A-I promoter is also observed with insulinomimetics such as bisperoxo (1,10-phenathroline) oxovanadate (bpv) and the protein kinase C (PKC) activator phorbol ester (PDBu) (17). However, insulin sensitization with thiazolidendiones may not always be sufficient to induce apo A-I expression (18,19). It appears that insulin acts on the apo A-I promoter through at least two signaling pathways: Ras-raf and phosphatidylinositol 3-kinase (PI3-K), leading to activation of the mitogen-activated protein kinase (MAPK) and PKC kinases, respectively (20). Despite these differences, all these pathways ultimately target the transcriptional factor Sp1 (20,21). Cotransfection of Sp1 expression vector with an apo A-I IRCE reporter construct augments the actions of insulin, while reducing Sp1 levels with an antisense RNA impairs the insulin response (17). In addition, experiments with okadaic acid (a cellular phosphatase inhibitor) or other phosphatase inhibitors suggest that phosphorylation of Sp1 plays a critical role in apo A-I expression by regulating the ability of Sp1 to bind the IRCE. The various molecular signals for Sp1-mediated apo A-I transcription are summarized in Fig. 1 (21). Indeed, numerous domains at Sp1 are targets for phosphorylation by multiple protein serine/threonine kinases. Mutations at threonine 266 to alanine have been shown to inhibit growth factor–mediated induction of apo AI gene expression (22).

EFFECT OF KETOACIDOSIS ON APO A-I EXPRESSION

One of the complications commonly found in subjects with uncontrolled type1 diabetes is ketoacidosis. Apo A-I gene expression is repressed during ketoacidosis. This change could be correlated with the process of ketogenesis but not with ketone bodies (23). In cell culture studies with hepatocytes and intestinal cells, ketones and their analogs such as butyrate or isobutyramide do not significantly alter apo A-I expression (24). In contrast, acidosis, induced either by changes in the pH of the culture media or by treatment with agents known to alter intracellular pH, consistently suppresses apo A-I promoter activity and apo A-I production (25). These changes were observed in both HepG2 cells and Caco2 cells, a human intestinal epithelial cell line that retains the ability of intestinal cells to produce apo A-I. The effect of acidosis on apo A-I promoter activity was specific and could not be explained by a nonspecific toxic effect of acidosis on protein synthesis (25).

The apo A-I downregulation with acidosis occurs through a pH-responsive element (pH-RE) located within the promoter (25). Acidosis increases the specific DNA binding activity of a putative repressor protein. This pH-RE contains an nTRE and is located 3′ and adjacent to the apo A-I TATA box (15,25,26). The pH-RE identified in HepG2 cells, using transient transfection analysis of the apo A-I promoter with deletion and site-directed mutations, overlaps the apo A-I TATA element (27). This region also contains binding sites (underlined) for other transcription factors including the thyroid hormone receptor (15), δ-EF1 (δ-crystallin enhancer binding protein-1) (28), and AP-1 (activator protein-1) (29) (Table 2). Repression of apo A-I promoter action by acidosis is not altered with cyclohexamide or by actinomycin D treatment, suggesting that this process does not require de novo protein or mRNA synthesis (25). Inhibition of tyrosine kinase activity and diacylglycerol-stimulated PKC signaling with tyrophosphotin A47 and phorbol myristate acetate (PMA), respectively, does not affect the repressive effect of acidosis on apo A-I promoter (25). This is somewhat surprising since acidosis has been shown to alter PKC activation, and others have shown that tyrosine kinase activity is necessary for acidosis to stimulate immediate-early gene expression in renal epithelial cells (30). It is possible that repression by acidosis requires more than one signaling pathway, one of which may involve PKC or tyrosine kinase activity.

Overall it is clear that ketoacidosis, primarily through changes in intracellular pH, suppresses apo A-I gene expression through inhibition of promoter activity. However, not all models of ketosis show repression. For example, apo A-I mRNA and protein levels are elevated in rats placed on a high-fat ketogenic diet (24). This indicates that acidosis is a required element of apo A-I gene suppression by ketoacidosis.

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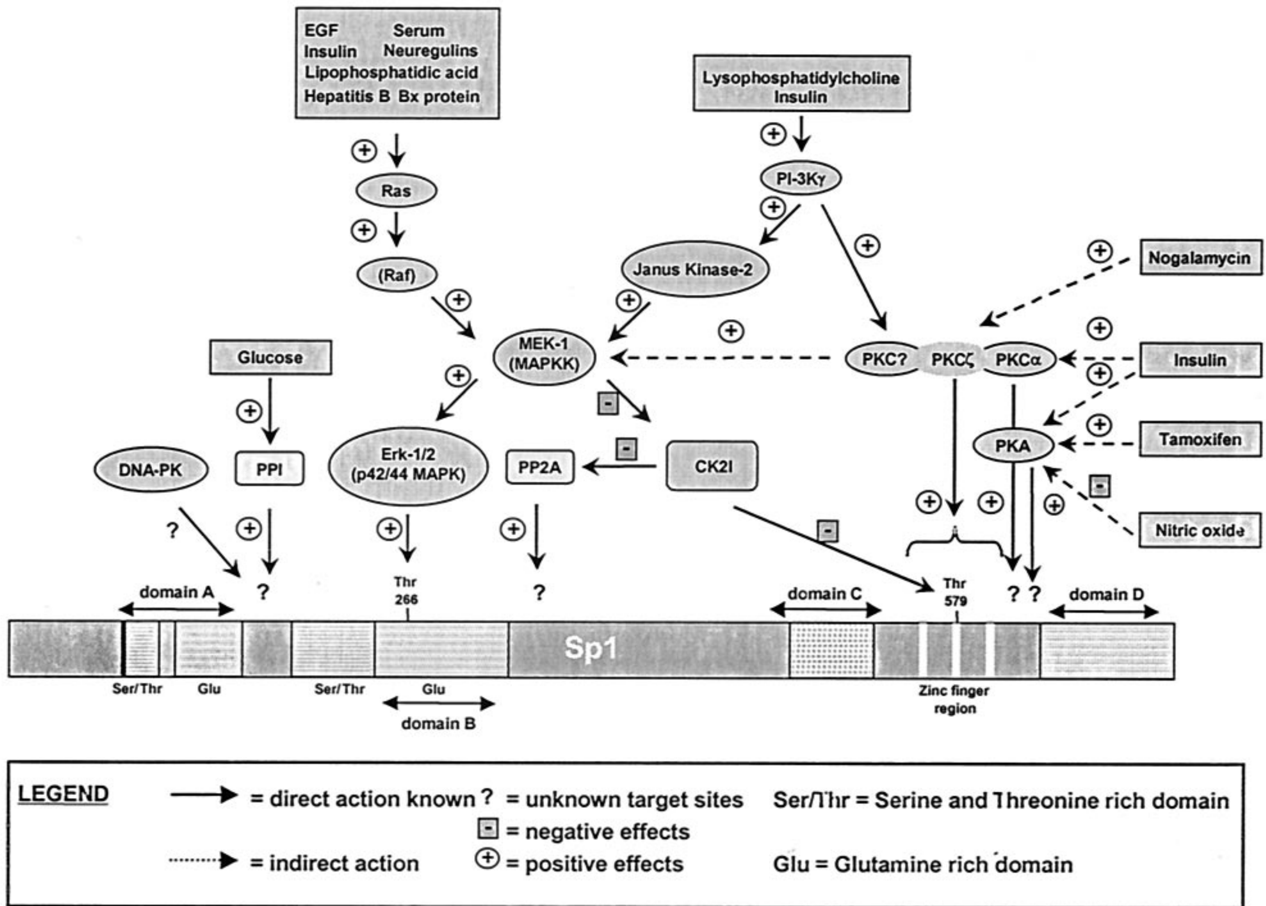


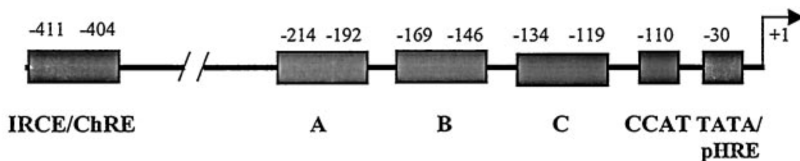
FIG. 1. The multiplicity of signaling pathways of Sp1-mediated modulation of gene expression. The figure is reproduced from ref. 21, with permission from the Society for Endocrinology.

EFFECT OF INSULIN RESISTANCE ON APO A-I EXPRESSION

One of the hallmarks of insulin resistance or the metabolic syndrome X is reduced plasma concentration of HDL cholesterol and low plasma levels of its major apoprotein, namely apo A-I (31–33). This change is mostly attributed to increased fractional clearance of HDL without a change in apo A-I production (32,33). However, it is possible that reduced apo A-I expression due to reduced responsiveness of the apo A-I gene to insulin may contribute to the reduced plasma concentrations of HDL.

The potential mediators of insulin resistance in obesity include increased plasma free fatty acid (FFA) concentrations or increased muscle and hepatic tissue content of triglycerides, increased production of leptin, tumor necrosis factor-α (TNF-α), and possible other yet unidentified humoral mediators (34).

The experimental models of insulin resistance and their effects on the apo A-I gene expression are summarized in Table 3. Because FFAs have been implicated as one of the causes of insulin resistance, we studied the effect of FFAs on insulin-stimulated apo A-I promoter activity in HepG2



IRCE/ChRE – Sp1

Site A – TR, HNF-4, RARα,β, RXRα, C/EBP, ARP-1, PPARα, Rev-erbα

Site B – Glucocorticoids, Estradiol, HNF3β

Site C – HNF-4, ARP-1

TATA – Basal transcriptional apparatus, TR, pH

FIG. 2. Organization of regulatory elements within the apo A-I gene promoter. Several regulatory elements mediating numerous transcriptional responses (hormonal, metabolic, and tissue-specific), as well as the factors that mediate both positive and negative effects on apo A-I gene expression, are shown. In some cases (estradiol, glucocorticoids, and pH), the promoter region modulating the effect has been identified but the factors mediating the process have not been reported. A cytokine response element mediating the suppressive effects of TNF-α and IL-1β is located within site A. TR, thyroid hormone receptor; IRCE/ChRE, insulin response core element/carbohydrate response element; pHRE, pH response element.

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TABLE 2
pH-RE binding proteins

Motif	Transcription factor	Ref.
acataTATAggtcaggg	"TATA" binding protein	27
acatatatAGGTCAggg	nTRE half-site	26
acatatatAGGTcaggg	δ-EF1	28
acatatatagGTCAggg	AP-1	29

The pH-RE identified in HepG2 cells using transient transfection analysis of apo A-I promoter with deletion and site-directed mutations overlaps with the apo A-I TATA element. This region also contains binding sites (underlined) for other transcription factors, including the thyroid hormone receptor, δ-EF1, and AP-1.

cells (35). The results indicate that FFA treatment abolishes both insulin and Sp-1–stimulated activation of apo A-I promoter. However, basal apo A-I gene expression was not altered in the presence of FFAs. The FFA stearic acid had no appreciable effect on Sp1-DNA binding (35). Therefore, stearic acid must affect Sp1 function through a mechanism unrelated to regulation of Sp1-DNA binding, most likely by altering posttranslational modification (35).

Another potential mediator of insulin resistance is leptin. Cohen et al. (36) found that leptin antagonizes insulin signaling by decreasing insulin-induced tyrosine phosphorylation of insulin receptor substrate (IRS)-1 and thereby causing a state of insulin resistance (37). Plasma leptin levels correlate with body adiposity, and we recently reported that hyperleptinemia occurs also in diet-induced models of insulin resistance (38,39). However, plasma leptin levels in diabetic subjects do not significantly differ from nondiabetic control subjects when corrected for differences in body fat content (40). In addition, the *ob/ob* mice, which lack leptin, develop insulin resistance with the onset of obesity (41). This suggests that there are factors other than leptin that contribute to the obesity-related hyperinsulinemia and insulin insensitivity. In our unpublished studies, we have been unable to demonstrate a significant effect of leptin on apo A-I gene expression in HepG2 cells under normal culture conditions. Thus, treatment of HepG2 cells with leptin over a wide concentration range (0–100 ng/ml) did not alter apo A-I promoter activity, apo A-I protein synthesis, or apo A-I mRNA levels (unpublished observations).

Finally, TNF-α, a pluripotent cytokine, is implicated in obesity-related hyperinsulinemia/insulin resistance (42, 43). Obese individuals tend to have increased plasma TNF-α levels (44,45), and in a study of a homogenous Native Canadian population, circulating TNF-α concentrations were positively correlated with the degree of insulin resistance (45). Previously published studies as well as our own observations show that TNF-α significantly downregulates apo A-I gene expression in HepG2 cells (46). There is some evidence that Sp1 activity is regulated by TNF-α. Pan et al. (47) reported that insulin treatment of rat hepatoma cells increases Sp1 and Sp3 accumulation by approximately threefold. However, exposure to TNF-α decreases Sp1 level to 50% of control values. In the same study, hepatic Sp1 levels were reduced in diabetic rats relative to control rats but could be normalized with insulin treatment (47). We have recently found that both TNF-α and interleukin (IL)-1β reduce apo A-I gene expression in a dose-dependent fashion (46). This inhibitory effect occurred at the transcriptional level. Treatment with TNF-α and IL-1β resulted in a dose-dependent reduction in apo A-I promoter activity. Using a series of promoter deletion constructs, this effect was found to be mediated through a cytokine responsive element within site A (46).

The role of prostanoids in mediating insulin action and in modulating the apo A-I expression has not been well characterized. Prostanoids are implicated in insulin signaling in the liver (48,49). Therefore, it is possible that interference with prostanoid production within the liver may alter insulin effects on apo A-I expression. In addition, some prostanoids are ligands of peroxisome proliferator–activated receptors (PPARs) (50,51), and PPARs are implicated in the regulation of apo A-I expression (52,53). Thus, it is possible that prostanoids may also have a direct role in modulating apo A-I expression. Recent work in our laboratory shows that cyclooxygenase (COX) inhibition with indomethacin or acetyl salicylic acid downregulates apo A-I protein and mRNA expression at the transcriptional level (54). This effect could not be attributed to either arachidonic acid excess or to a deficiency in various prostanoids tested, including prostaglandin I_2, thromboxane B_2, (±) 5-HETE or (±) 12-HETE, and prostaglandin E_1 and E_2 (54).

The precise underlying mechanism of indomethacin-related downregulation of apo A-I expression is not known. The observed changes could be related to COX inhibition or could be independent of COX inhibition. Although normal hepatocytes do not express COX, it is possible that inhibition of COX activity in nonparenchymal hepatic cells, such as Kupfer cells, could alter paracrine signaling.

It is noteworthy that aspirin, but not indomethacin, has been shown to decrease the expression of apo a in human hepatocytes at the transcriptional level (55). This effect was independent of COX inhibition. It is possible that both the apo A-I and apo a genes have aspirin responsive regions in their promoter. Large clinical studies are

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TABLE 3
Experimental models of insulin resistance and their effects on apo A-I gene expression both in cultured cells and in vivo

Model	Effect on apo A-I gene expression	Ref.
FFAs	Suppression of insulin-mediated induction of apo A-I gene expression in vitro by saturated fatty acids only	35
Leptin	No effect on basal or insulin-induced apo A-I gene expression	Unpublished
Fructose feeding	Induction of apo A-I gene expression in vivo	56
Inflammatory cytokines	Suppress apo A-I gene expression at the transcriptional level (TNF-α and IL-1β)	46
Aging	Induction of apo A-I gene expression in rats in vivo	57
Glucosamine	Increased apo A-I mRNA stabilization	58

needed to address the clinical relevance of the effects of aspirin on apo A-I and apo a expression.

The reduced expression of apo A-I in obese type 2 diabetic subjects, despite the increased levels of plasma insulin, cannot be simply attributed to insulin resistance. Thus, insulin resistance and hyperinsulinemia induced with a high-fructose diet in rats is associated with increased apo A-I levels (56). Similarly, aging in rats is associated with increased expression of apo A-I, although it is accompanied with insulin resistance (57).

Finally, treatment of hepatocytes with glucosamine, a model of cellular insulin resistance, is associated with increased stabilization of apo A-I mRNA and increased expression of apo A-I protein (58).

It appears that the diabetes-related reduction in apo A-I is not only related to increased plasma clearance of the protein but also is the result of downregulation of apo A-I expression at a transcriptional level. Although the precise nature for this change is not known, it is likely that increased cytokine production may contribute significantly to the inhibition of apo A-I gene transcription.

EFFECTS OF MICRONUTRIENTS ON APO A-I EXPRESSION

Uncontrolled hyperglycemia is commonly associated with alterations in micronutrient status (59). Some minerals, such as chromium, vanadium, magnesium, and zinc, have either insulinomimetic effects or have permissive effects on insulin action. Deficiencies in some minerals may occur in poorly controlled diabetic subjects. In addition to their effects on insulin action, some micronutrients have been found to have an important modulatory role in apo A-I gene expression (60,61). In particular, zinc deficiency causes downregulation of apo A-I expression (59), whereas supraphysiologic concentrations of zinc, as well as chromium or vanadium, downregulate apo A-I promoter activity (61). Some of the effects of zinc deficiency may be explained by the dependence of zinc finger–containing transcription factors, such as Sp1, for the coordinating effect of zinc ions.

Vitamins, notably those with antioxidative potential, may affect apo A-I expression. The apo A-I promoter is sensitive to the oxidative state (61), and some antioxidants at high concentrations can suppress apo A-I promoter activity in Hep G2 cells (62). Clinical trials have also found that antioxidants may partially blunt HDL induction by simvastatin-niacin combination therapy (63). The precise role of micronutrient status in the diabetes-related reduction of apo A-I expression is not known. It is conceivable that when a significant deficiency state occurs, it may aggravate the lowered plasma HDL concentrations.

CONCLUSIONS

The cause of increased cardiovascular morbidity and mortality in diabetic subjects is not entirely clear. Observational studies suggest that it may be related to insulin resistance and associated comorbidities, including hypertension, diabetes, and an atherogenic plasma lipid profile (1).

One of the hallmarks of insulin resistance or the metabolic syndrome X is reduced plasma concentration of HDL cholesterol and low plasma levels of its major apoprotein, namely apo A-I (31). Diabetic subjects with the metabolic syndrome have significantly increased risk of cardiovascular disease compared with diabetic subjects who do not have the metabolic syndrome (64). The reduced plasma levels of apo A-I in this syndrome is mostly attributed to increased fractional clearance of HDL (32,33). However, it is possible that reduced apo A-I expression due to either reduced responsiveness of the apo A-I gene to insulin, or secondary to inhibition by the altered metabolic milieu in diabetes, notably increased cytokine production, may contribute to the reduced plasma concentrations of HDL. In addition, in type 1 diabetic subjects who experience frequent ketoacidosis, the apo A-I gene transcriptional activity is further compromised through a transcriptional repressor protein that interacts with a pH-RE within the promoter. The myriad of changes that may occur in diabetes, culminating in reduced plasma apo A-I concentrations, is summarized in Fig. 3. Future studies elucidating molecular determinants of apo A-I gene expression should help in the rational design of novel therapeutic targets to increase HDL level and reduce cardiovascular disease in diabetes.

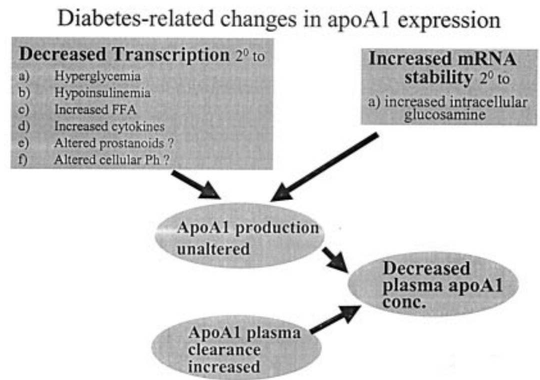

FIG. 3. A schematic summary of the myriad of changes that occur in diabetes, culminating in reduced plasma apo A-I concentrations.

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REFERENCES

1. Mooradian AD: Cardiovascular disease in type 2 diabetes mellitus: current management guidelines. *Arch Intern Med* 163:33–40, 2003
2. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M: Mortality from coronary heart disease in subjects with type 2 diabetes and in non-diabetic subjects with and without prior history of myocardial infarction. *N Engl J Med* 339:229–234, 1998
3. Garg A, Grundy SM: Management of dyslipidemia in NIDDM. *Diabetes Care* 13:153–169, 1990
4. Turner RC, Millns H, Neil HA, Stratton IM, Manley SE, Matthews DR, Holman RR: Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS 23). *BMJ* 316:823–828, 1998
5. Kawahiri M, Maugeais C, Rader D: High-density lipoprotein metabolism: molecular targets for new therapies for atherosclerosis. *Curr Atheroscler Rep* 2:363–372, 2000
6. Rubins HB, Robins SJ, Collins D: Gemfibrozil for the secondary prevention of coronary heart disease in men with low levels of high-density lipoprotein cholesterol: Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial Study Group. *N Engl J Med* 341–410-418, 1999
7. Gordon T, Castelli WP, Hjotland MC: High density lipoprotein as a protective factor against coronary heart disease: the Framingham study. *Am J Med* 62:707–714, 1977
8. Berliner JA, Navab M, Fogelman AM: Atherosclerosis: basic mechanisms: oxidation, inflammation, and genetics. *Circulation* 91:2488–2496, 1995
9. Anderson L: Pharmacology of apolipoprotein A-I. *Curr Opin Lipidol* 8:225–228, 1997
10. Eriksson M, Carlson LA, Miettinen TA, Angelin B: Stimulation of fecal

steroid excretion after infusion of recombinant proapolipoprotein A-I: potential reverse cholesterol transport in humans. *Circulation* 100:594–598, 1999
11. Ginsberg HN: Diabetic dyslipidemia: basic mechanisms underlying the common hypertriglyceridemia and low HDL cholesterol levels. *Diabetes* 45 (Suppl. 3):S27–S30, 1996
12. Hargrove GM, Junco A, Wong NCW: Hormonal regulation of apolipoprotein AI. *J Mol Endocrinol* 22:103–111, 1999
13. Taylor AH, Raymond J, Dionne JM, Romney J, Chan J, Lawless DE, Wanke IE, Wong NCW: Glucocorticoid increases rat apolipoprotein AI promoter activity. *J Lipid Res* 37:2232–2243, 1996
14. Harnish DC, Evans MJ, Scicchitano MS, Bhat RA, Karathanasis SK: Estrogen regulation of the apolipoprotein AI promoter through transcription factor sharing. *J Biol Chem* 273:9270–9278, 1998
15. Taylor AH, Wishart P, Lawless DE, Raymond J, Wong NCW: Identification of functional positive and negative thyroid hormone-responsive elements in the rat apolipoprotein A_1 promoter. *Biochem* 35:8281–8288, 1996
16. Murao K, Wada Y, Nakamura T, Taylor AH, Mooradian AD, Wong NCW: Effects of glucose and insulin on rat apolipoprotein A-I gene expression. *J Biol Chem* 273:18959–18965, 1998
17. Lam JK, Matsubara S, Mihara K, Zheng X, Mooradian AD, Wong NCW: Insulin induction of apolipoprotein A: role of SP1. *Biochemistry* 42:2680–2690, 2003
18. Mooradian AD, Haas MJ, Wong NCW, Chehade JH: Apolipoprotein A-I expression in rats is not altered by troglitazone. *Exp Biol Med* 227:1001–1005, 2002
19. Sakamoto J, Kimura H, Moriyama S, Odaka H, Momose Y, Sugiyama Y, Sawada H: Activation of human peroxisome proliferator-activated receptor (PPAR) subtypes by pioglitazone. *Biochem Biophys Res Commun* 278:704–711, 2000
20. Zheng XL, Matsubara S, Diao C, Hollenberg MD, Wong NC: Activation of apolipoprotein AI gene expression by protein kinase A and kinase C through transcription factor, Sp1. *J Biol Chem* 275:31747–31754, 2000
21. Samson SL, Wong NC: Role of Sp1 in insulin regulation of gene expression. *J Mol Endocrinol* 29:265–279, 2002
22. Zheng XL, Matsubara S, Diao C, Hollenberg MD, Wong NC: Epidermal growth factor induction of apolipoprotein A-I is mediated by the Ras-MAP kinase cascade and Sp1. *J Biol Chem* 276:13822–13829, 2001
23. Haas MJ, Pun K, Reinacher D, Wong NCW, Mooradian AD: Effects of ketoacidosis on rat apolipoprotein A1 gene expression: a link with acidosis but not with ketones. *J Mol Endocrinol* 25:129–139, 2000
24. Haas MJ, Reinacher D, Pun K, Wong NCW, Mooradian AD: Induction of the apolipoprotein A1 gene by fasting: a relationship with ketosis but not with ketone bodies. *Metabolism* 49:1572–1578, 2000
25. Haas MJ, Reinacher D, Li JP, Wong NCW, Mooradian AD: Regulation of ApoA1 gene expression with acidosis: requirement for a transcriptional repressor. *J Mol Endocrinol* 27:43–57, 2001
26. Romney JS, Chan J, Carr FE, Mooradian AD, Wong NCW: Identification of the thyroid hormone responsive mRNA-S_{11} as apolipoprotein A_1 mRNA and the effects of the hormone on the promoter. *Mol Endocrinol* 6:943–950, 1992
27. Sastry KN, Seedorf U, Karathanasis SK: Different *cis*-acting DNA elements control expression of the human apolipoprotein AI gene in different cell types. *Mol Cell Biol* 8:605–614, 1988
28. Sekido R, Murai K, Funahashi JI, Kamachi Y, Fujisawa-Sehara A, Nabeshima YI, Kondoh H: The δ-crystalline enhancer-binding protein δ-EF1 is a repressor of E2-box-mediated gene activation. *Mol Cell Biol* 14:5692–5700, 1994
29. Rao GN, Katki KA, Madmanchi NR, Wu Y, Birrer M: J Jun B forms the majority of the AP-1 complex and is a target for redox regulation by receptor tyrosine kinase and G protein-coupled receptor agonists in smooth muscle cells. *J Biol Chem* 274:6003–6010, 1999
30. Yamaji Y, Moe OW, Tyler R, Alpern RJ: Acid activation of immediate early genes in renal epithelial cells. *J Clin Invest* 94:1297–1303, 1994
31. Lopez-Candales A: Metabolic syndrome X: a comprehensive review of the pathophysiology and recommended therapy. *J Med* 32:283–300, 2001
32. Vajo Z, Terry JG, Brinton EA: Increased intra-abdominal fat may lower HDL levels by increasing the fractional catabolic rate of Lp A-I in postmenopausal women. *Atherosclerosis* 160:495–501, 2002
33. Duvillard L, Pont F, Florentin E, Gambert P, Verges B: Inefficiency of insulin therapy to correct apolipoprotein A-I metabolic abnormalities in non-insulin-dependent diabetes mellitus. *Atherosclerosis* 152:229–237, 2000
34. Mooradian AD: Obesity: a rational target for managing diabetes mellitus. *Growth Hormone IGF Res* (Suppl. A):S1–S5, 2001
35. Haas MJ, Horani MH, Wong NCW, Mooradian AD: Saturated fatty acids repress insulin-induction of the apolipoprotein AI gene promoter through inhibition of Sp1 activity. In *Endocrine Society 85th Annual Meetings*. Bethesda, MD, Endocrine Society, 2003, p. 135
36. Cohen B, Novick D, Rubinstein M: Modulation of insulin activities by leptin. *Science* 274:1185–1188, 1996
37. Taylor SI, Barr V, Reitman M: Does leptin contribute to diabetes caused by obesity? *Science* 274:1151–1152, 1996
38. Mooradian AD, Chehade J, Hurd R, Haas MJ: Monosaccharide-enriched diets cause hyperleptinemia without hypophagia. *Nutrition* 16:439–441, 2000
39. Mooradian AD, Chehade J: Serum leptin response to endogenous hyperinsulinemia in aging rats. *Mech Ageing Devel* 115:101–106, 2000
40. McGregor GP, DeSaga JF, Ehlenz K, Fischer A, Heese F, Hegele A, Lammer C, Pensel C, Lang RE: Radioimmunological measurement of leptin in plasma of obese and diabetic human subjects. *Endocrinology* 137:1501–1504, 1996
41. Burant CF, Sreenan S, Hirano KI: Troglitazone action is independent of adipose tissue. *J Clin Invest* 100:2900–2908, 1997
42. Hotamisligil GS, Shargill NS, Spiegelman BM: Adipose expression of tumor necrosis factor-alpha: direct role in obesity linked insulin resistance. *Science* 259:87–91, 1993
43. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM: IRS-1 mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha and obesity-induced insulin resistance. *Science* 271:665–668, 1996
44. Dandona P, Weinstock R, Thusu K: Tumor necrosis factor-alpha in sera of obese patients: fall with weight loss. *J Clin Endocrinol Metab* 83:2907–2910, 1998
45. Zinman B, Hanley AJ, Harris SB, Kwan J, Fantus IG: Circulating tumor necrosis factor alpha concentrations in a Native Canadian population with high rate of type 2 diabetes mellitus. *J Clin Endocrinol Metab* 84:272–278, 1999
46. Haas MJ, Horani MH, Wong CNW, Mooradian AD: Suppression of apolipoprotein A-I gene expression in HepG2 cells by TNF α and IL 1β. *Biochim Biophys Acta* 1623:120–128, 2003
47. Pan X, Solomon SS, Borromeo DM, Martinez-Hernandez A, Raghow R: Insulin deprivation leads to deficiency of Sp1 transcription factor in H-411E hepatoma cells and in streptozotocin-induced diabetic ketoacidosis in the rat. *Endocrinology* 142:1635–1642, 2001
48. Wasner HK, Weber S, Partke HJ, Amini-Hadi-Kiashar H: Indomethacin treatment causes loss of insulin action in rats: involvement of prostaglandins in the mechanism of insulin action. *Acta Diabetologica* 31:175–182, 1994
49. Christensen JR, Hammond BJ, Smith GD: Indomethacin inhibits endocytosis and degradation of insulin. *Biochem Biophys Res Commun* 173:127–133, 1990
50. Willson TM, Brown PJ, Sternbach DD, Heake BR: The PPARs: from orphan receptors to drug discovery. *J Med Chem* 43:527–550, 2000
51. Dussault I, Forman BM: Prostaglandins and fatty acids regulate transcriptional signaling via the peroxisome proliferator activated receptor nuclear receptors. *Prostaglandins Other Lipid Mediat* 62:1–13, 2000
52. Lefebre AM, Peinado-0nsurke J, Leitersdorf I, Briggs MR, Paterniti JR, Fruchart JC, Fievet C, Auwerx J, Staels B: Regulation of lipoprotein metabolism by thiazolidinediones occurs through a distinct but complementary mechanism relative to fibrates. *Arterio Thromb Vasc Biol* 17:1756–1764, 1997
53. Vu-Dac N, Chopin-Delannoy S, Gervois P, Bonnelye E, Martin G, Fruchart J-C, Laudet V, Staels B: The nuclear receptors peroxisome proliferator-activated receptor α and Rev-erb α mediate the species-specific regulation of apolipoprotein A_1 expression by fibrates. *J Biol Chem* 273:25713–25720, 1998
54. Horani M, Gobal F, Haas MJ, Wong NCW, Mooradian AD: Cyclooxygenase (COX) inhibition is associated with downregulation of apolipoprotein AI (Apo-AI) promoter activity in cultured hepatoma cell line-HepG2. *Metabolism*. In press
55. Kagawa A, Azuma H, Akaike M, Kanagawa Y, Matsumoto T: Aspirin reduces apolipoprotein (a) (apo(a)) production in human hepatocytes by suppression of apo (a) gene transcription. *J Biol Chem* 274:34111–34115, 1999
56. Mooradian AD, Wong NCW, Shah GN: Apolipoprotein A1 expression in young and aged rats is modulated by dietary carbohydrates. *Metabolism* 46:1132–1136, 1997
57. Shah NG, Wong NCW, Mooradian AD: Age-related changes in apolipoprotein A-I expression. *Biochim Biophys Acta* 1259:277–282, 1995
58. Haas MJ, Wong NCW, Mooradian AD: Effect of glucosamine on apolipoprotein AI mRNA stabilization and expression in HepG2 cells. *Metabolism*. In press
59. Franz MJ, Bantle JP, Beebe CA, Brunzell JD, Jean-Louis Chiasson J-L, Garg A, Holzmeister LA, Hoogwerf B, Mayer-Davis E, Mooradian AD, Purnell JQ,

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Wheeler M: Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications (Technical Review). *Diabetes Care* 25:148–198, 2002
60. Wu JY, Wu Y, Reaves SK, Wang YR, Lei PP, Lei KY: Apolipoprotein A-I gene expression is regulated by cellular zinc status in hep G2 cells. *Am J Physiol* 277:C537–C544, 1999
61. Haas MJ, Sawaf R, Horani MH, Gobal F, Wong NCW, Mooradian AD: Effect of chromium on apolipoprotein A-I expression in HepG2 cells. *Nutrition* 19:353–357, 2003
62. Haas MJ, Wadud K, Wong NCW, Mooradian AD: Antioxidants suppress the apolipoprotein AI (apoAI) promoter in the cultured hepatoma cell line, HepG2. In *Endocrine Society 84th Annual Meetings*. Bethesda, MD, Endocrine Society, 2002, p. 262
63. Cheung MC, Zhao X-Q, Chait A, Albers JJ, Brown G: Antioxidant supplements block the response of HDL to simvastatin-niacin therapy in patients with coronary artery disease and low HDL. *Arterioscler Thromb Vasc Biol* 21:1320–1326, 2001
64. Alexander CM, Landsman PB, Teutsch SM, Haffner SM, Third National Health and Nutrition Examination Survey (NHANES III) National Cholesterol Education Program (NCEP): NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. *Diabetes* 52:1210–1214, 2003

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