High-Density Lipoprotein Modulates Glucose Metabolism in Patients With Type 2 Diabetes Mellitus

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Background—Low plasma high-density lipoprotein (HDL) is associated with elevated cardiovascular risk and aspects of the metabolic syndrome. We hypothesized that HDL modulates glucose metabolism via elevation of plasma insulin and through activation of the key metabolic regulatory enzyme, AMP-activated protein kinase, in skeletal muscle.

Methods and Results—Thirteen patients with type 2 diabetes mellitus received both intravenous reconstituted HDL (rHDL: 80 mg/kg over 4 hours) and placebo on separate days in a double-blind, placebo-controlled crossover study. A greater fall in plasma glucose from baseline occurred during rHDL than during placebo (at 4 hours rHDL = −2.6±0.4; placebo = −2.1±0.3 mmol/L; P = 0.018). rHDL increased plasma insulin (at 4 hours rHDL = 3.4±10.0; placebo = −19.2±7.4 pmol/L; P = 0.034) and also the homeostasis model assessment β-cell function index (at 4 hours rHDL = 18.9±5.9; placebo = 8.6±4.4; P = 0.025). Acetyl-CoA carboxylase β phosphorylation in skeletal muscle biopsies was increased by 1.7±0.3-fold after rHDL, indicating activation of the AMP-activated protein kinase pathway. Both HDL and apolipoprotein AI increased glucose uptake (by 177±12% and 144±18%, respectively; P < 0.05 for both) in primary human skeletal muscle cell cultures established from patients with type 2 diabetes mellitus (n = 5). The mechanism is demonstrated to include stimulation of the ATP-binding cassette transporter A1 with subsequent activation of the calcium/calmodulin-dependent protein kinase kinase and the AMP-activated protein kinase pathway.

Conclusions—rHDL reduced plasma glucose in patients with type 2 diabetes mellitus by increasing plasma insulin and activating AMP-activated protein kinase in skeletal muscle. These findings suggest a role for HDL-raising therapies beyond atherosclerosis to address type 2 diabetes mellitus. (Circulation. 2009;119:2103-2111.)

Key Words: glucose ■ insulin ■ lipoproteins ■ metabolism ■ muscles
acid muscle, AMPK activation generates ATP by increasing fatty

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Data are mean ± SD obtained from initial screening. HbA1c indicates glycated hemoglobin; OGTT, oral glucose tolerance test.

major apolipoprotein, apolipoprotein AI (apoAI), activate the key metabolic regulatory enzyme AMP-activated protein kinase (AMPK) in endothelial cells and are critical for the nitric oxide–mediated vasodilatory effects of HDL.8 In skeletal muscle, AMPK activation generates ATP by increasing fatty acid β-oxidation through activation of acetyl Co-A carboxylase β (ACCβ) and by increasing glucose uptake. AMPK is regulated by nutritional status, exercise, and adipokines9 and plays a pivotal role in skeletal muscle glucose disposal.

Recent studies have demonstrated that infusions of recombinant and reconstituted HDL (rHDL) have modest effects on coronary plaque morphology and volume10,11 and improve endothelial function in type 2 diabetes mellitus.12 However, the potential for HDL-raising agents to modulate glucose metabolism remains unclear. We hypothesized that a 4-hour infusion of rHDL would reduce plasma glucose through mechanisms including modulation of plasma insulin and activation of AMPK in skeletal muscle. This was tested in a randomized, crossover, double-blind, placebo-controlled study in patients with type 2 diabetes mellitus. We further sought to characterize potential novel mechanisms contributing to HDL-mediated glucose disposal in murine β-cell and primary human skeletal muscle cell culture experiments.

### Methods

#### Human rHDL Clinical Trial

Thirteen patients with type 2 diabetes mellitus participated in the study (Table 1), which was approved by the Alfred Hospital Ethics Committee and performed in accordance with the Declaration of Helsinki. All patients provided written, informed consent. Diabetic status was confirmed with the use of standard criteria (fasting plasma glucose >7.1 mmol/L or a 2-hour blood glucose level of >11.1 mmol/L after a 70-g oral glucose load [oral glucose tolerance test]). Those with a history of major illness were excluded. Seven patients were unmedicated, 4 were treated with gliclazide, and 1 each was treated with glibenclamide and glimepiride (withdrawn for 5 half-life periods before participation). No other medications were used by any of the patients, and medication use had no systematic effect on any of the parameters assessed.

#### Study Design: rHDL Infusion

Participants received rHDL (80 mg/kg over 4 hours; CSL Behring AG, Bern, Switzerland) and saline placebo on separate occasions separated by at least 4 weeks in a double-blind crossover study. After an overnight fast, participants arrived at the laboratory at 8 AM, and catheters were inserted into antecubital veins in both arms for administration of treatment (rHDL or placebo) and blood sampling (every 30 minutes during the infusion). A percutaneous muscle biopsy of the vastus lateralis muscle was performed under local anesthesia before (0 hour) and after (4 hours) infusion on both occasions.

#### rHDL Constituents and Preparation

See the online-only Data Supplement.

#### Plasma Analyses

See the online-only Data Supplement.

#### Muscle Biopsy Analyses

See the online-only Data Supplement.

#### Murine Pancreatic β-Cell Studies

Insulin secretion was assessed in murine β-cells (MIN6N8). Cells were grown to subconfluence, then placed in normal glucose media (5 mmol/L) with treatments (oxidized LDL 50 μg/ml, HDL 50 μg/ml) for 72 hours. Insulin secretion into the media after brief (20 minutes) and sustained (2 hours) glucose stimulus (20 mmol/L) was measured by enzyme-linked immunosorbent assay. Data are expressed as the percentage of insulin detected in the media (as a function of total insulin in both the media and cells) during either the brief or sustained phase of glucose-induced secretion.

#### Human Primary Skeletal Muscle Cell Culture Studies

#### Skeletal Muscle Cell Culture

Five male volunteers (Table 2) with type 2 diabetes mellitus underwent a percutaneous vastus lateralis muscle biopsy (~120 mg) from which satellite myoblasts were isolated.13,14 Skeletal muscle cultures were treated with physiological concentrations of human HDL (50 μg/ml), apoAI (40 μg/ml), and LDL (50 μg/ml) as described in the online-only Data Supplement. Other treatments included insulin (100 mmol/L) and the AMPK activator phenformin (1 mmol/L) administered as a positive control.

#### Lipoprotein Isolation

See the online-only Data Supplement.

#### Glucose Uptake

Glucose uptake was determined by the 2-deoxy-glucose method.15 Nonspecific uptake was assessed with the use of cytochalasin-B (10 μmol/L), which was subtracted from total uptake.

#### Fatty Acid Oxidation

The oxidation of fatty acids was measured by the uptake and conversion of labeled [1-14C]palmitic acid into 14CO₂ and acid-soluble metabolites as described previously.14
AMPKα and ACCβ Phosphorylation
See the online-only Data Supplement.

AMPK Activity
See the online-only Data Supplement.

Dominant Negative Studies
AMPK was inhibited via an adenovirus delivered dominant negative AMPKα1 (AMPK-DN) mutant as described previously.16 The virus was titrated to maximize protein expression without visible morphological change to cells, resulting in a multiplicity of infection of 300 with AMPK-DN or empty vector (AdGO) 72 hours before treatment or assays.

LKB1 Activity
See the online-only Data Supplement.

Intracellular Ca2+
Intracellular calcium was measured by Fura-2 as described in the online-only Data Supplement.

Calcium/Calmodulin-Dependent Protein Kinase
Kinase Studies
Skeletal muscle cells were pretreated with the calcium/calmodulin-dependent protein kinase (CaMKK) inhibitor STO609 (Sigma, St Louis, Mo) at 2 µmol/L for 1 hour or cyclopiazonic acid at 10 µmol/L for 15 minutes, and other treatments were then applied as described above. Cells were washed before lysing and Western blotting.

ABCA1-Blocking Antibodies
A specific anti-ABCA1-blocking antibody, NDF4C2 (4C2), was employed to examine whether ABCA1 is involved in HDL/apoAI-mediated glucose uptake in skeletal muscle. This antibody has been characterized previously in the setting of macrophages and monocytes to reduce cholesterol efflux.17 The antibody was incubated with cells at 20 µg/mL for 18 hours, and data were compared by incubation with a nonspecific isotype control antibody (IgM).17

Antibodies
See the online-only Data Supplement.

Statistical Analysis
Normally distributed data were compared by t tests or repeated-measures ANOVA with least significant difference post hoc tests used to compare individual means as appropriate. The order of the rHDL and placebo infusions was included as a between-subjects variable in the analysis of the clinical studies. Nonnormally distributed data were compared by Mann-Whitney rank sum tests or Kruskal-Wallis 1-way ANOVA on ranks with the Dunn post hoc tests to compare individual means as appropriate. Results are expressed as mean±SEM unless otherwise indicated. All analyses were conducted with the use of SPSS (version 16) and Sigmastat (version 3.5). Cell culture data represent a minimum of 4 separate cell lines, and all assays were performed in triplicate. A P value of <0.05 was considered significant.

Results
Human rHDL Clinical Trial
rHDL infusion increased plasma HDL by 1.33±0.43-fold (Figure 1A) and plasma apoAI protein (Figure 1B) by 2.41±0.15-fold (P<0.001 for both) compared with placebo at the end of the 4-hour infusion. No significant liver function abnormalities or other adverse effects associated with rHDL were found (data not shown).

Plasma glucose declined throughout both interventions consistent with fasting status (Figure 2A). The reduction from baseline was significantly greater after rHDL compared with placebo (placebo, −2.17±0.34 versus rHDL, −2.57±0.40 mmol/L; 10 of 13 patients had a greater decrease during rHDL; P=0.018; Figure 2A). It is noteworthy that these results were achieved in a short time frame (4 hours) and despite a high degree of heterogeneity within the study population with respect to the severity of diabetes mellitus (fasting glucose ranged from 8 to 17 mmol/L). The increase in plasma insulin from baseline was greater after rHDL than placebo (12 of 13 patients had a greater increase during rHDL; P=0.034; Figure 2B). The homeostasis model assessment (HOMA) for β-cell function index was elevated from placebo after rHDL infusion, suggesting enhanced β-cell function (10 of 13 patients had a greater increase during rHDL; P=0.05 from 120 minutes; Figure 2C). No effect was found on HOMA insulin resistance index throughout either infusion (Figure 2D).

To further explore the effect of rHDL infusion on skeletal muscle glucose disposal mechanisms, activation of the AMPK pathway was examined in vastus lateralis biopsies. Although AMPK itself is usually only transiently activated (see “Human Primary Skeletal Muscle Cell Culture Studies”), the downstream target, ACC, typically exhibits a more sustained activation.8,18,19 Consistent with this pattern, rHDL did not influence AMPK activity or phosphorylation at Thr-172 (data not shown) after the 4-hour infusion, but ACCβ phosphorylation was significantly elevated 1.7±0.33-fold after rHDL compared with placebo infusion (0.81±0.11-fold relative to baseline).

Murine Pancreatic β-Cell Studies
Incubation of cultured murine β-cells with oxidized LDL for 72 hours reduced insulin secretion (Figure 3A), and cotreat-
ment with HDL reversed this response (Figure 3B). HDL also significantly increased the acute phase glucose-stimulated insulin secretion in cultured /H9252-cells (Figure 3C). These changes in insulin secretion were observed in the absence of changes in insulin gene and protein expression (proinsulin; data not shown), supporting the contention that HDL stimulates insulin secretion.

Human Primary Skeletal Muscle Cell Culture Studies

HDL and ApoAI Increase Glucose Uptake and Fatty Acid Oxidation in Primary Human Skeletal Muscle Cells

HDL significantly increased glucose uptake in cultured skeletal muscle cells by 178±12% (P<0.001) at a dose of 50 μg/mL (Figure 4A and 4B). Interestingly, apoAI, the major apolipoprotein of HDL, also significantly increased glucose uptake (by 144±17%; P=0.02; Figure 4B). Neither the phospholipid component of HDL nor LDL induced an increase in glucose uptake (data not shown and Figure 4A, respectively). The magnitude of the effect of HDL and apoAI on glucose uptake in type 2 diabetic cells was comparable with insulin (Figure 4B). Further evidence for activation of the AMPK pathway was demonstrated by an increase in palmitate oxidation in HDL- and apoAI-treated cells (141±13%, P=0.02; 150±9%, P=0.02, respectively) compared with untreated cells (Figure 4C).

HDL/ApoAI Activates AMPK In Vitro

HDL and apoAI transiently increased the phosphorylation of AMPK at Thr172 after 5-minute treatments, an effect that dissipated by 30 minutes (Figure 4D). Increases in AMPK phosphorylation were similar to that achieved by a 30-minute treatment with phenformin, a known activator of AMPK (Figure 4D). Increased phosphorylation of AMPKα coincided with increased AMPKα2 activity after 30-minute treatments with phenformin (2.22±0.35-fold; P=0.001) and 5-minute treatments with both HDL (1.77±0.18-fold; P<0.001) and apoAI (1.9±0.14-fold; P=0.002; Figure 4E). AMPKα1 activity was unchanged (data not shown). Increases in AMPKα2 activity were associated with elevated ACCβ phosphorylation in response to both HDL and apoAI incubation for 15 minutes (188±5%, P<0.001 and 151±16%, P=0.006, respectively) and 30 minutes (250±66%, P<0.001 and 255±70%, P<0.05 for both, respectively; Figure 4F). These results indicate a delayed yet prolonged phosphorylation of ACC, in contrast to the phosphorylation of AMPK, which is consistent with the findings of our clinical study.

Infection of skeletal muscle cells with an AMPK-DN virus resulted in successful elevation of AMPKα1 protein, with no change in AMPKα2 (Figure 5A). HDL- and apoAI-stimulated glucose uptake was abolished in the presence of AMPK-DN virus compared with empty virus (AdGO) (from 170±28% to 106±10%, P=0.004 and 142±14% to 91±13%, P=0.02, respectively; Figure 5B). The observed increase in fatty acid oxidation after treatment with both HDL and apoAI was also abolished in the presence of AMPK-DN virus (from 146±8% to 78±16% and 139±6% to 76±14%, respectively; P=0.01; Figure 5C).

Figure 2. Effect of rHDL infusion on metabolic parameters. Change from baseline (0 hours) in plasma glucose (A), plasma insulin (B), HOMA β-cell function (C), and HOMA insulin resistance index (D) during rHDL and placebo infusions are shown. *Differences between treatments (P<0.05, repeated-measures ANOVA) for A, B, C, and D.
HDL Induces Calcium Release and Activates CaMKK

The activity of LKB1, a common upstream kinase of AMPK, was not significantly altered by incubation with HDL or apoAI (Figure 6A). Further investigations implicated elevated intracellular calcium and activation of CaMKK as mechanisms upstream of AMPK.

HDL increased skeletal muscle intracellular calcium by almost 3-fold and returned to basal levels within 2 minutes (Figure 6B). The magnitude of the response was comparable to that elicited by calcium ionophore (Figure 6B). Furthermore, HDL-induced phosphorylation of AMPK was completely abolished in the presence of the CaMKK inhibitor STO609 compared with vehicle, implicating CaMKK in the HDL-induced activation of AMPK (Figure 6C).

HDL/ApoAI Modulates Skeletal Muscle Glucose Metabolism in an ABCA1-Dependent Manner

The presence of the ABCA1 receptor was confirmed in skeletal muscle plasma membrane preparations, and its expression was upregulated by treatment with the liver-X-receptor agonist TO-901317 (2 μmol/L, 18 hours) (Figure 7A). Cholesterol efflux from cultured skeletal muscle to HDL and apoAI has been demonstrated previously and was confirmed in the present investigation (Figure I in the online-only Data Supplement), attesting to the functionality of the ABC class of receptors in this tissue. The ABCA1-blocking antibody 4C2 significantly inhibited the HDL- and apoAI-induced glucose uptake (Figure 7B) and also completely inhibited HDL-induced Ca²⁺ release (Figure 7C). However, treatment with 4C2 had no effect on cholesterol efflux (Figure I in the online-only Data Supplement), suggesting activation of a signaling pathway unrelated to cholesterol efflux via ABCA1.

Discussion

The present study comprehensively demonstrates that HDL has the potential to modulate glucose metabolism in humans with type 2 diabetes mellitus. Short-term elevation of plasma HDL in patients with type 2 diabetes mellitus reduced plasma glucose. This reduction likely occurs through increased glucose disposal via mechanisms including elevated plasma insulin and activation of AMPK in skeletal muscle. These findings suggest a potential role for HDL-raising therapies beyond vascular disease to address key aspects of the metabolic syndrome.

The relative contribution of elevated plasma insulin and activation of the AMPK pathway to the reduction in plasma glucose cannot be determined. However, it is notable that the effects on plasma glucose were observed as early as 30 minutes into the 4-hour rHDL infusion, whereas the effects on insulin were not apparent until 2.5 hours. Furthermore, when one considers that study participants had insulin-resistant type 2 diabetes with HOMA insulin resistance indices ranging from 3 to 12 (average at baseline 4.9; values >2.6 are considered insulin resistant), the plasma glucose response to insulin elevation would have been attenuated. This suggests that the plasma glucose reduction may have resulted primarily from activation of the AMPK pathway. Although the magnitude of the plasma glucose reduction might be considered modest, it is likely of clinical significance given (1) that patients were fasted, (2) the short-term nature of the stimulus, and (3) the numerous homeostatic pathways controlling blood glucose.

Although the insulin elevation may have had a lesser contribution to the glucose reduction during the rHDL infusion than AMPK, HDL may nevertheless represent an important insulin-modulating mechanism. The mouse β-cell data corroborate the clinical observation of insulin elevation and...
provide preliminary evidence that HDL directly stimulates insulin secretion from pancreatic β-cells. This contention is supported by other recent β-cell studies reporting HDL-mediated insulin secretion via ABCA1 and reversal of oxidized-LDL–induced reductions in β-cell insulin secretion by HDL.

Demonstration that HDL elevation activates the AMPK pathway in skeletal muscle in humans is a novel finding. It is supported by a recent study suggesting that apoAI phosphorylates AMPK in murine skeletal muscle in vitro, but the clinical relevance of this finding is unclear given the marked differences in cholesterol metabolism between rodents and humans. In the clinical trial, we demonstrate activation of the AMPK pathway by rHDL. Furthermore, activation of the AMPKα2 isoform (and not α1) by HDL in cell culture experiments is consistent with the pattern elicited by moderate cycling exercise, leptin, and adiponectin, highlighting physiological relevance. The dependence of HDL-mediated glucose uptake on AMPK is further substantiated by the AMPK-DN data.

Together with evidence suggesting that apoAI/HDL can transcytose through endothelial layers and thus contact skeletal muscle, the actions of HDL on glucose uptake may occur through a direct receptor-mediated event. HDL and apoAI mediate other processes such as reverse cholesterol transport via cell surface proteins including the ABC class of transporters (eg, ABC-A1, -G1, -G4, -G5). The present data indicate that in addition to mediating reverse cholesterol transport, HDL also initiates a calcium-sensitive signaling cascade through ABCA1. This is consistent with previous observations that HDL increases intracellular calcium in endothelial cells. The increase in calcium stimulates CaMKK, which we show phosphorylates and activates AMPK. This is consistent with recent studies demonstrating that CaMKK activates AMPK in skeletal muscle in response to other stimuli. Given that skeletal muscle is the major site of glucose utilization in the body (~80%), this mechanism is likely of potential significance for whole body glucose homeostasis and may contribute to the observed plasma glucose reduction in the rHDL clinical study.

**Limitations**

The conclusions may be restricted to patients with type 2 diabetes mellitus, which is the patient population for whom
the data have greatest therapeutic relevance. This study does not definitively determine whether the rHDL-induced elevation in plasma insulin was a result of increased insulin secretion. However, when considered in the context of the murine \textit{H}9252\textit{c}ell experiments and previous literature,\textsuperscript{5,6} the circumstantial evidence is compelling. Furthermore, the clinical investigation does not permit delineation of the respective contribution of elevated insulin and activation of skeletal muscle AMPK to the reduction in plasma glucose. Finally, whether long-term elevation of HDL causes sustained metabolic effects is a question that should be addressed as long-term HDL-raising agents become clinically available.

**Clinical Implications**

Although no studies have appeared that directly examine the metabolic actions of HDL, the present findings are consistent with clinical observations that low HDL precedes development of type 2 diabetes mellitus and that some agents that raise HDL improve glucose metabolism and prevent diabetes mellitus. Blood lipid and lipoprotein levels, including low HDL, are known to be important predictors for development of type 2 diabetes mellitus. Statins increased HDL and not only reduced the development of new type 2 diabetes mellitus in a large interventional trial\textsuperscript{32} but also contributed to improved insulin sensitivity after short-term treatment,\textsuperscript{33} although such effects are not universal.\textsuperscript{34} Additionally, bezafibrate, a peroxisome proliferator-activated receptor-\(\alpha\) agonist, increased circulating HDL levels in conjunction with reductions in plasma glucose and delayed the onset of type 2 diabetes mellitus in a population with impaired fasting glucose.\textsuperscript{35} Finally, exercise training is tightly linked with improved glycemic control and elevation of circulating HDL.\textsuperscript{4,36}

Given that HDL elevation with statins, fibrates, and exercise is quite modest (\(\sim 20\%\)), it is interesting to speculate on the potential of specific HDL-raising therapies that may elicit a sustained increase in HDL by 50\% to 100\%\textsuperscript{11,37,38} to influence glucose metabolism. Although the first large clini-

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**Figure 5.** HDL-induced glucose uptake and fatty acid oxidation is AMPK dependent. A, Western blot for AMPK\textsubscript{\(\alpha\)1} in cells infected with increasing doses (multiplicity of infection [MOI]) of either AMPK-DN virus or empty virus (AdGO). B, Glucose uptake in skeletal muscle cells infected with empty virus (AdGO) or AMPK-DN virus (DN) stimulated with 50 \(\mu\text{g/mL}\) HDL or 40 \(\mu\text{g/mL}\) apoAI for 1 hour. C, Palmitate oxidation in skeletal muscle cells infected with empty virus or AMPK-DN virus stimulated with HDL or apoAI for 2 hours. All lipoproteins were derived from healthy pooled plasma. *Significant effect of AMPK-DN (n=4; \(P<0.01\), unpaired \(t\) test).

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**Figure 6.** HDL modulates AMPK through a calcium-dependent pathway. A, LKB1 activity after treatment with vehicle, 50 \(\mu\text{g/mL}\) HDL, or 40 \(\mu\text{g/mL}\) apoAI for 5 minutes (n=3). B, Top, Representative tracing for Fura-2 ratio indicating points at which treatments were added (HDL, 30 seconds; ionophore [Iono], 130 seconds). Mean change in intracellular free calcium (Ca\(^{2+}\)) as measured by Fura-2 radiometric dye in response to HDL and ionophore (n=4). C, Phosphorylated (Thr-172) AMPK after 1-hour pretreatment with STO-609 or vehicle and stimulation with Ca\(^{2+}\) ionophore, cyclopiazonic acid (CPA), and HDL (n=5). All lipoproteins were derived from healthy pooled plasma. #Significant difference between treatment and vehicle; *significant differences from control; \(P<0.05\), 1-way ANOVA and least significant difference post hoc analysis.
In conclusion, these data support the hypothesis that HDL/apoAI reduces glucose uptake by increasing intracellular free calcium (Ca^{2+}) that activates glucose transporters (GLUTs). The increase in GLUT4 expression and glucose uptake in response to HDL/apoAI is consistent with previous studies indicating that HDL/apoAI decreases plasma glucose by increasing plasma insulin and improves glucose metabolism. These findings have implications beyond vascular disease to managing type 2 diabetes mellitus.

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Acknowledgments

References


CLINICAL PERSPECTIVE

Low plasma high-density lipoprotein (HDL) is an important risk factor for cardiovascular disease. Low HDL has also been associated with metabolic syndromes, whereas high HDL associated with physical training occurs in conjunction with enhanced glucose tolerance. Despite such associations, this is the first clinical study to describe a mechanistic link between HDL and glucose metabolism. The primary observation of reduced plasma glucose after short-term HDL elevation is underpinned by at least 2 mechanisms: elevated plasma insulin and enhanced muscle glucose uptake via AMP-activated protein kinase activity in human skeletal muscle. Biochem Biophys Res Commun. 2000;273:1150–1155.


31. Haffner SM, Stern MP, Hazuda HP, Mitchell BD, Patterson JK. Cardio-


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SUPPLEMENTAL MATERIAL

METHODS:

Human rHDL clinical trial

*rHDL constituents and preparation*

rHDL consists of apolipoprotein AI (apoAI) isolated from human plasma and phosphatidylcholine (PC) from soy bean. ApoAI and PC are combined in the presence of sodium cholate in a molar ratio of 1:150 and form disc-shaped, non-covalently associated particles resembling nascent high density lipoproteins (HDL). Although this preparation does not fully reflect a complex composition of mature HDL,\(^1\) upon infusion rHDL undergoes rapid remodelling and/or interaction with host HDL\(^2\) making it likely that its composition becomes similar to that of mature HDL. This preparation produces biological responses analogous to native HDL in previous studies.\(^3\) Protein electrophoresis demonstrated that apoAI was the dominant protein in both rHDL and the native HDL used to treat cell cultures, and that these preparations did not contain any other proteins (leptin, insulin, adiponectin) likely to induce a metabolic response (data not shown).

*Plasma analyses*

Blood from participants was collected into appropriate anti-coagulant, plasma immediately separated by centrifugation and snap frozen. HDL-cholesterol was measured by colorimetric assay (WAKO Pure Chemical Industries, Osaka, Japan), apoAI protein by ELISA (Alerchek, Portland, USA), glucose by mass-spectroscopy (LC-MS)\(^4\) and insulin by RIA (Millipore, Billerica, MA, USA). Beta cell function and insulin resistance index were calculated using the homeostasis model assessment (HOMA).\(^5\)
Muscle biopsy analyses

Biopsy samples were immediately snap frozen in liquid nitrogen. Before analysis, frozen samples were placed in lysis buffer and immediately homogenised and clarified by centrifugation. Protein analysis was performed as described below.
Human primary skeletal muscle cell culture studies

Lipoprotein isolation

Human HDL (d=1.12-1.21g/mL) and LDL (d=1.063-1.085g/mL) were isolated from whole, pooled plasma from healthy individuals by ultracentrifugation, as previously described. Lipid-free apoAI was isolated and purified from HDL by size exclusion chromatography following delipidation with chloroform-ethanol, according to the method described previously. Presence of phospholipid in the isolated apoAI was not detectable using the Bartlett phospholipid assay after repeated chloroform-methanol extraction. The concentrations of all lipoproteins and their derivatives added to cultures were based on protein content.

AMPKα and ACCβ phosphorylation

Skeletal muscle cells were serum deprived for 4hrs then treated for designated times with either phenformin, insulin, HDL or apoAI. Cells were washed and lysed in protein lysis buffer and western blotting performed as previously described.

AMPK activity

Cells were serum deprived for 4hrs then treated with either HDL or apoAI for 5mins or phenformin for 30mins. AMPK activity was determined in the presence of 200µM AMP as described previously.

LKB1 activity

Cells were serum deprived for 4hrs then treated with either HDL or apoAI for 5mins. LKB1 activity was determined as described previously.

Intracellular Ca^{2+}

Skeletal muscle cells were incubated in the presence of either the calcium ionophore, A23187 (10µM) to directly elevate Ca^{2+} levels, or the sarcoplasmic reticulum (SR) Ca^{2+} pump inhibitor,
cyclopiazonic acid (CPA, 10µM). CPA inhibits the SR Ca\(^{2+}\) pump resulting in increased cytoplasmic Ca\(^{2+}\). Cells were plated onto glass coverslips and grown and treated as described above. Intracellular Ca\(^{2+}\) concentration was estimated by fluorescence spectroscopy using Fura-2 AM (Molecular Probes) and a ratiometric fluorescence imaging system (Ionoptix, Boston, MA), with excitation performed at 340 and 380 nm and emission monitoring at 505 nm. Fluorescence data were expressed as the 340/380 ratio after subtraction of background auto-fluorescence.

**Antibodies**

Anti-human apoAI monoclonal antibodies were generated at the Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia.\(^{11}\) Anti-phospho Thr-172 AMPK\(\alpha\), Ser-221 ACC\(\beta\), AMPK\(\alpha\)1/2 and LKB1 polyclonal antibodies were generated as previously described.\(^{12}\) Anti-leptin antibody was from R&D systems and anti-insulin and anti-adiponectin antibodies were from Chemicon. Anti-total CaMKK (\(\alpha\) and \(\beta\)) was from BD transduction laboratories. Anti-\(\beta\)-actin was from Cell Signaling Technologies. ABCA1 antibodies were generated at the Baker IDI Heart and Diabetes Institute. Both anti-mouse and anti-rabbit HRP conjugated secondary antibodies were from Amersham Biosciences (Buckinghamshire, UK).
References


Figure Legends

Figure 1; Effects of the ABCA1 blocking antibody (4C2) on cholesterol efflux from skeletal muscle cells.
Cholesterol efflux was measured as the amount of $^3$H-cholesterol effluxed into media as a percentage of total cholesterol in both media and cells. Treatments, HDL (10µg/ml from pooled plasma) or rHDL (10µg/ml), were applied after incubation with antibodies (Isotype control or ABCA1 blocking, 4C2) for 18hrs. Background values (i.e. the efflux in the absence of HDL) were subtracted from all values. n=5.

Figure 2; Schematic diagram of the mechanisms by which HDL is postulated to modulate plasma glucose.
HDL is postulated to increase peripheral glucose uptake and reduce plasma glucose by both increasing plasma insulin (possibly by increased insulin secretion) and via the ABCA1 receptor and stimulation of CaMKK to activate the AMPK pathway in skeletal muscle.
Figure 1

- Isotype Control Antibody
- ABCA1 Blocking Antibody

Cholesterol Efflux (%)

Cont | HDL | rHDL

0  | 5  | 10

15
Figure 2

HDL

↑ insulin

PANCREAS

↑ insulin

OTHER PERIPHERAL TISSUES?

PLASMA GLUCOSE

↓

SKELETAL MUSCLE

↑ Ca²⁺

CaMKK

AMPK

ABCA1