Type 2 Diabetes Is Associated with Reduced ATP-Binding Cassette Transporter A1 Gene Expression, Protein and Function

Dipesh C. Patel 1,*, Christiane Albrecht 4, Darrell Pavitt 1, Vijay Paul 3, Celine Pourreyron 5, Simon P. Newman 2, Ian F. Godsland 1, Jonathan Valabhji 1, Desmond G. Johnston 1

1 Division of Medicine, Imperial College London, London, United Kingdom, 2 Oncology Drug Discovery and Women’s Health Group, Faculty of Medicine, Imperial College London, London, United Kingdom, 3 Department of Physiology, Technical University of Munich, Munich, Germany, 4 Institute of Biochemistry and Molecular Medicine, University of Bern, Bern, Switzerland, 5 Centre of Oncology and Molecular Sciences, University of Dundee, Dundee, United Kingdom

**Abstract**

**Objective**: Increasing plasma glucose levels are associated with increasing risk of vascular disease. We tested the hypothesis that there is a glycaemia-mediated impairment of reverse cholesterol transport (RCT). We studied the influence of plasma glucose on expression and function of a key mediator in RCT, the ATP binding cassette transporter-A1 (ABCA1) and expression of its regulators, liver X receptor-α (LXRα) and peroxisome proliferator-activated receptor-γ (PPARγ).

**Methods and Results**: Leukocyte ABCA1, LXRα and PPARγ expression was measured by polymerase chain reaction in 63 men with varying degrees of glucose homeostasis. ABCA1 protein concentrations were measured in leukocytes. In a sub-group of 25 men, ABCA1 function was quantified as apolipoprotein-A1-mediated cholesterol efflux from 2–3 week cultured skin fibroblasts. Leukocyte ABCA1 expression correlated negatively with circulating HbA1c and glucose (rho = −0.41, p < 0.001; rho = −0.34, p = 0.006 respectively) and was reduced in Type 2 diabetes (T2DM) (p = 0.03). Leukocyte ABCA1 protein was lower in T2DM (p = 0.03) and positively associated with plasma HDL cholesterol (HDL-C) (rho = 0.34, p = 0.02). Apolipoprotein-A1-mediated cholesterol efflux correlated negatively with fasting glucose (rho = −0.50, p = 0.01) and positively with HDL-C (rho = 0.41, p = 0.02). It was reduced in T2DM compared with controls (p = 0.04). These relationships were independent of LXRα and PPARγ expression.

**Conclusions**: ABCA1 expression and protein concentrations in leukocytes, as well as function in cultured skin fibroblasts, are reduced in T2DM. ABCA1 protein concentration and function are associated with HDL-C levels. These findings indicate a glycaemia-related, persistent disruption of a key component of RCT.

**Introduction**

People with hyperglycaemia are at increased risk of coronary heart disease (CHD). The risk is high in type 2 diabetes (T2DM) (2–4 fold elevation) [1] and applies even to those with lesser degrees of glucose elevation (impaired glucose regulation). Even in the general population, there is a positive relationship between glucose (or HbA1c) levels and CHD rates.[2] Several underlying mechanisms have been proposed, including protein glycation and free radical damage.[3] Both diabetes and impaired glucose regulation are accompanied by dyslipidaemia, a major feature of which is low levels of circulating high density lipoprotein cholesterol (HDL-C). Impaired reverse cholesterol transport (RCT) has therefore been implicated.[4]

RCT is the process by which excess cholesterol is removed from the body. It begins in peripheral tissues when lipid-poor apolipoprotein A-I (apo-A1) induces cholesterol and phospholipid mobilisation from intracellular storage sites to the plasma membrane.[5] The membrane-associated ATP binding cassette transporter-A1 (ABCA1) is integral to the subsequent transmembrane lipid transfer to form nascent HDL.[6] Later cholesterol efflux to HDL is mediated by the related transporter, ATP binding cassette transporter-G1 (ABCG1) with some evidence of synergism.[7] Defects in ABCA1 impair apo-A1-mediated lipid efflux from cells and ABCA1 knockout mice develop early atherosclerosis.[8,9] Functional ABCA1 mutations in both Tangier disease and familial HDL deficiency lead to very low levels of circulating HDL, almost all of which is lipid-poor, as newly synthesised apo-A1 fails to acquire cholesterol and phospholipids.[10]

ABCA1 expression in several tissues is up-regulated by oxysterol interaction with the liver X receptor-α (LXRα). Stimulation of LXRα transcriiption by peroxisome proliferator-activated receptor-γ (PPARγ) upregulates ABCA1 expression and increases apo-A1-mediated cholesterol efflux from macrophages.[11]

ABCA1 expression is decreased in the liver and peritoneal macrophages of diabetic compared to control mice and protein
levels have been reported to be reduced in mouse models of diabetes.[12,13] In human studies, fibroblast ABCA1 function has been shown to be impaired by advanced glycation end products [14] and we have previously observed an inverse relationship between ABCA1 expression in peripheral leukocytes and fasting glucose in healthy young and middle-aged men.[15] These findings suggested a potential mechanism for the hyperglycaemia-induced increased risk of early vascular disease. In the present study, we explore relationships between glycaemia, expression of ABCA1 and ABCG1, ABCA1 protein concentrations and ABCA1 function in people with varying degrees of hyperglycaemia, and whether these relationships are influenced by LXRα or PPARγ expression.

Methods

Participants

Sixty three men aged over 18 years were recruited from regional metabolic clinics and primary care, with the assistance of the UK Diabetes Research Network. Participants included 18 with T2DM and 18 with impaired glucose regulation by World Health Organization criteria. The patients with T2DM were treated with diet alone and had not taken hypoglycaemic or lipid-lowering medication. Subjects taking systemic corticosteroids or medications with marked effects on insulin secretion were excluded. Patients were free of microvascular and macrovascular complications. 27 healthy, aged matched normoglycaemic controls were also studied following local advertisement.

Ethics statement

Written consent was obtained and ethical approval for the study was from the Hammersmith, Queen Charlotte’s and Chelsea Research Ethics Committee (REC Reference 05/Q0406/33). The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Procedures

After an overnight fast, a clinical and routine biochemical assessment was conducted. The control subjects and those without known diabetes underwent a 75g Oral Glucose Tolerance Test (OGTT) and were classified using the American Diabetes Association criteria (2003).[16] Fasting blood samples only were taken from patients with known T2DM. 25 participants, selected randomly, consented to undergo more detailed investigation which involved a 4 mm punch skin biopsy sample from the forearm under sterile conditions to enable functional analyses in skin fibroblasts.

Laboratory measurements

Fasting glucose, creatinine and liver enzymes were measured using standard laboratory techniques. HbA1c (DCCT aligned) was measured by cation-exchange high performance liquid chromatography using a Tosoh Automated glycohaemoglobin analyser G7 (Tosoh Bioscience Inc., Tokyo, Japan). Cholesterol, lipoprotein and triglyceride measurements were performed using an Olympus AU 2700 autoanalyser (Olympus Life & Material Science, Ireland). LDL-cholesterol was calculated using the Friedewald formula. Plasma non-esterified fatty acid (NEFA) concentrations were measured by an additional housekeeper, glyceraldehyde 3-phosphate dehydrogenase (GAPDH). As results with GAPDH were very similar to those with β-Actin, only β-Actin derived data are described. Target gene quantity was calculated from a standard curve. Gene expression data from multiple experiments were compared to a calibrator sample and normalised to the housekeeper gene.

Leukocyte protein expression

Total cell lysates were prepared by incubating the leukocyte cell pellets (n = 50) with 1 ml ice-cold lysis buffer (50 mM mannitol, 2 mM EDTA, 50 mM, pH 7.6) containing complete protease inhibitor (Roche, Germany) and 0.1% w/v Triton X-100 for 60 minutes on ice. This was followed by five homogenization cycles (20 s each and 10 minutes resting time on ice after each homogenization step) in a Fastprep homogenizer. The leukocyte homogenates were centrifuged at 15,000 g for 15 minutes at 4°C and the supernatants containing clear cell lysates were collected. Protein concentrations were determined by a bicinchoninic acid assay.[17] The clear leukocyte cell lysates were stored at −80°C until ABCA1 protein measurement was performed. Frozen leukocyte lysates were thawed and ABCA1 protein concentration was measured using an ELISA method as previously described.[18] Briefly, either 50 µl ABCA1 peptide (ab14148, Abcam, UK) calibrators (range 8–1000 ng) or 50 µl of unknown leukocyte lysates were incubated in a ABCA1 peptide pre-coated and BSA blocked micro titre plate along with 100 µl (2 µg/l diluted in PBST) anti-ABCA1 rabbit antibody (ab57360, Abcam, UK). The incubation was carried out for 5 hours at room temperature while shaking on an ELISA plate shaker. The plate was washed with PBST and further processed by using biotin labelled anti-rabbit IgG goat antibody, streptavidin-HRP conjugate and tetramethylbenzidine enzyme substrate system. The enzymatic reaction was stopped by addition of sulphuric acid and absorbance measured at 450 nm on an ELISA plate reader (Sunrise, Tecan, Austria). ABCA1 protein concentrations of unknown samples were calculated from a calibration curve and results were expressed as ABCA1 protein ng/µg total protein.

Fibroblast cell culture and cholesterol efflux measurement

Primary fibroblast cell lines were obtained from human dermal biopsy following enzymatic digestion in the sub-group of 25 subjects (9 controls, 6 with impaired regulation and 10 T2DM) who underwent a skin biopsy. Primary cells were cultured with DMEM (Invitrogen Ltd, Paisley, UK) which contained 25 mmol/l glucose, supplemented with 10% fetal bovine serum and 1% antimicrobial solution (Sigma-Aldrich Co, Poole, UK). The medium was changed twice weekly until cells were 80-100% confluent. After culture for 2–3 weeks, cells from passage 4 to 7 were used for cholesterol efflux measurements. Fibroblasts were plated in wells until 60–80% confluence. Cholesterol loading and
efflux experiments were carried out following an accepted methodology using apo-A1.[19] In brief, cells were loaded with 0.5 μCi/ml 1,2-3[H]-cholesterol (Perkin Elmer, Boston, MA) for 24 hours in the presence of 30 μg/ml unlabelled cholesterol (Sigma-Aldrich Co.) to induce ABCA1. Cells were then washed with PBS/BSA 0.2% (v/v) and efflux was commenced using 10 μg/ml apo-A1 (Sigma-Aldrich Co.). After 20 hrs the medium was collected, centrifuged and radioactivity was measured using liquid scintillation counting (Beckman LS6000 Coulter UK Ltd, High Wycombe, UK). Cellular cholesterol was extracted using hexane/propanol (3:2 v/v) and counted. Percentage cholesterol efflux was calculated by dividing the radioactive counts in the efflux medium by the sum of the counts in the media and cell extract. Experiments were performed in triplicate with a mean CV of 12%. Apo-A1-mediated lipid export was derived by subtraction of non-specific efflux (measured in control wells without apo-A1).

**Statistical analysis**

Variation between the groups was compared by ANOVA, or Kruskal-Wallis test depending on variable distribution. Likewise, a t-test or Mann-Whitney test was used to compare individual groups. Smoking status was analysed using a Chi squared test. Relationships between variables were assessed by Spearman correlation. Multiple linear regression was used to assess independence of relationships after log-transformation, where appropriate, to normalise distributions. A two sided p value <0.05 was considered significant. Sigmasstat version 3.5 (Systat Software, Inc.) and Prism version 4 (Graphpad Software, Inc.) were used for analyses and graphical illustration, respectively.

**Results**

As expected, patients with diabetes were heavier compared to controls and had a greater waist circumference (Table 1). HDL-C was significantly lower in patients with diabetes, compared with controls, but other lipid parameters were not significantly different across subject groups. Standard laboratory renal, liver and thyroid function tests were normal in all subjects. HbA1c and fasting glucose have been used as the indices of glycaemia as they were measured in all subjects and they correlated strongly with each other (rho = 0.77, p<0.0001).

**ABCA1 gene expression and protein concentrations in peripheral blood leukocytes**

Leukocyte ABCA1 expression was significantly lower in patients with T2DM compared with controls (p = 0.03; Figure 1A). In the combined groups, ABCA1 expression fell with increasing HbA1c (rho = −0.41, p<0.001; Figure 2A) and with increasing fasting glucose levels (rho = −0.34, p = 0.006; Figure 2B).

There was no significant relationship between leukocyte ABCA1 expression and age, body mass index or waist circumference. Leukocyte ABCA1 protein concentration was lower in patients with T2DM compared with controls (p = 0.03; Figure 1B) although the relationship with fasting glucose was not significant statistically (rho = −0.25, p = 0.08). No significant relationship was observed between plasma NEFA levels and ABCA1 expression (p = 0.40).

**Relationship of ABCG1 expression with glycaemia and ABCA1 expression**

Leukocyte ABCA1 and ABCG1 expression were positively related (rho = 0.40, p = 0.005, Figure S1). Leukocyte ABCG1 expression in patients with T2DM was not significantly reduced (p = 0.08) and there were no differences between other groups. There was a borderline negative relationship between leukocyte ABCG1 expression and HbA1c (rho = −0.29, p = 0.05) Figure S1, but not fasting glucose (p = 0.33). Analysis of ABCG1 expression and glycaemia (mean glucose or HbA1c) using a linear model, demonstrated that only ABCA1 expression remained a significant predictor of ABCG1 expression.

**Apolipoprotein-A1 mediated cholesterol efflux in skin fibroblasts and glycaemia**

Apo-A1-mediated cholesterol efflux from fibroblasts was reduced by 36% in diabetes (p = 0.04; Figure 3A). After adjusting

### Table 1. Baseline participant demographic data.

| Variable                  | Controls (n = 27) | IGH (n = 18) | DM (n = 18) | ANOVA (p) |
|---------------------------|------------------|--------------|-------------|-----------|
| Age, yrs                  | 52±16            | 58±8         | 57±11       | 0.40      |
| BMI, kg/m²                | 24.3±2.2         | 27.3±4.1     | 27.9±3.2*   | <0.001    |
| Waist circumference, cm   | 91 (85–96)       | 96 (87–105)  | 101 (94–105)* | 0.003     |
| Alcohol consumption, units| 20 (4–24)        | 8 (2–18)     | 12 (2–20)   | 0.15      |
| Smokers, %                | 3 (10)           | 6 (33)       | 4 (22)      | 0.192     |
| Systolic BP, mmHg         | 132±13           | 138±17       | 131±14      | 0.30      |
| Diastolic BP, mmHg        | 81±10            | 83±11        | 82±6        | 0.77      |
| Fasting Glucose, mmol/l   | 5.10 (4.70–5.40) | 5.56 (4.95–5.95) | 6.8 (6.3–8.8) | <0.001    |
| HbA1c, %                  | 5.5±0.4          | 5.9±0.5      | 7.3±1.5†    | <0.001    |
| 2 hr Glucose, mmol/l      | 4.9±1.3          | 7.3±2.2*     | -           | <0.001    |
| Total Cholesterol, mmol/l | 5.38±0.89        | 5.14±1.06    | 5.03±1.10   | 0.49      |
| HDL-C, mmol/l             | 1.53±0.29        | 1.32±0.38    | 1.23±0.26*  | 0.005     |
| LDL-C, mmol/l             | 3.29±0.65        | 3.26±0.71    | 3.11±0.89   | 0.72      |
| Triglycerides, mmol/l     | 1.17 (0.74–1.59) | 0.97 (0.77–1.38) | 1.37 (0.94–2.11) | 0.18      |

Normally distributed variables are expressed as mean and standard deviation. Skewed data are represented by median (interquartile range). Abbreviations: IGH = impaired glucose regulation; DM = type 2 diabetes; ANOVA or equivalent analysis of variance; *p<0.05 relative to controls, †p<0.05 relative to control and IGH subjects.

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for protein concentration, efflux remained significantly lower in patients with diabetes compared to healthy men (4.5% versus 11.2%, p = 0.008). Efflux fell with increasing fasting plasma glucose (rho = 0.50, p = 0.01; Figure 3B). The inverse relationship with HbA1c was not statistically significant (rho = 0.30, p = 0.07). There was no significant relationship between apo-A1-mediated cholesterol efflux and age nor with body weight. Apo-A1-mediated cholesterol efflux from fibroblasts fell with increasing waist circumference (r = 0.53, p < 0.01) but bivariate analysis demonstrated this relationship to be dependent on fasting plasma glucose.

**Relationship of ABCA1 to plasma HDL cholesterol**

Leukocyte ABCA1 and ABCG1 expression did not significantly associate with plasma HDL-C concentrations. Leukocyte ABCA1 protein concentrations correlated positively and independently with circulating plasma HDL-C levels (rho = 0.34, p = 0.02; Figure 4A). Cholesterol efflux related positively to plasma HDL-C concentrations (rho = 0.41, p = 0.02; Figure 4B).

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**Figure 1.** *ABCA1* gene expression and protein concentration in blood leukocytes is reduced in type 2 diabetes. A. Leukocyte *ABCA1* gene expression, normalised to housekeeper gene (mean and standard error) according to subject glycaemic status in 63 participants. Con = Controls (n = 27), IGH = Impaired glucose regulation (n = 18), DM = type 2 diabetes (n = 18). Con vs. DM; p = 0.03, Con vs. IGH; p = 0.27, IGH vs. DM p = 0.33 (* p < 0.05). B. Leukocyte *ABCA1* protein concentration (ng/μg protein) expressed as mean and standard error is illustrated according to subject glycaemic status in 50 participants. Con = Controls (n = 17), IGH = Impaired glucose regulation (n = 17), DM = type 2 diabetes (n = 16) Con vs DM; p = 0.03, Con vs IGH; p = 1; IGH vs DM p = 0.06 (* p < 0.05).

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**Figure 2.** Leukocyte *ABCA1* gene expression is reduced with increasing glycaemia. A. Relationship of leukocyte *ABCA1* expression (normalised to housekeeper gene) to glycated haemoglobin (HbA1c) in 63 participants (27 control, 18 impaired glucose homeostasis and 18 type 2 diabetes) rho = −0.41, p < 0.001. B. Relationship of leukocyte *ABCA1* expression (normalised to housekeeper gene) to fasting plasma glucose in 63 participants (27 control, 18 impaired glucose homeostasis and 18 type 2 diabetes) rho = −0.34, p = 0.006.
We have demonstrated that ABCA1 gene expression, protein concentrations and transporter function are reduced in drug naive men with T2DM. Gene expression and protein concentrations were reduced in blood leukocytes, and cellular cholesterol removal to Apo-A1 (which exclusively involves ABCA1) was reduced in skin fibroblasts. These relationships were independent of variation in LXRα or PPARγ expression. These observations suggest novel mechanisms whereby hyperglycaemia could adversely influence cellular cholesterol metabolism. Such mechanisms could contribute to the well-established association between elevated glucose levels and risk of vascular disease.

ABCA1 is highly expressed in macrophages. Ideally, ABCA1 expression and protein content should be studied in arterial wall macrophages, but this was not possible for ethical reasons. Leukocyte ABCA1 RNA levels in humans reflect ABCA1 expression in circulating monocytes and can be used as a marker for this.[20] It should be noted that gene expression increases 4-fold in monocytes during differentiation into macrophages.[21] Peripheral blood leukocytes have the advantage of being readily obtained from large numbers of subjects and leukocyte ABCA1 has proven a useful surrogate in other clinical situations where the variations in ABCA1 expression have been observed in man.[22]

Leukocyte ABCA1 has not been previously considered to influence plasma HDL or RCT directly.[20,23] Recent data, however, have suggested that cells other than macrophages influence tissue cholesterol removal (eg adipocytes).[24] Leukocyte ABCA1 is known to protect against atherosclerosis in animals[25], but our observations cannot distinguish between possible direct and indirect effects of leukocyte ABCA1 on lipid transport.
ABCA1 expression was decreased in leukocytes from patients with T2DM and was directly related to the level of glycaemia. Most of the decline in gene expression occurred up to an HbA1c level of 7.5%, with little further fall above this value. Our ABCA1 expression data are compatible with and extend our previous findings in healthy men.[15] We further demonstrate concordant changes in leukocyte ABCA1 expression and protein concentrations in patients with T2DM. Our leukocyte expression results appear to contrast with the findings reported by Hoang and colleagues who did not find a difference in leukocyte ABCA1 expression between patients with diabetes and controls.[22] This previous study was conducted in a smaller cohort with one third of patients receiving hypoglycaemic medications. ABCA1 function was assessed on the basis of the ability of the subject’s plasma to induce cholesterol efflux from human macrophages cell lines.[22] There were baseline differences in lipid parameters which may have had an effect on the capacity of plasma to induce cholesterol efflux. [22] Our findings are compatible with previous data in monocytes, where reduced ABCA1 expression was observed in patients with diabetes and dyslipidaemia.[26]

Our results contrast with studies that showed reduced expression and function of ABCG1 (but not ABCA1) in monocytes and macrophages in people with T2DM [27,28], but these earlier studies were not designed or conducted in drug naive patients who were free of complications. In the study by Mauldin and colleagues [28], information pertaining to age, other medical conditions and drug treatments was unavailable. Forty percent of participants studied by Zhou et al., had evidence of retinopathy or nephropathy and all were receiving either oral hypoglycaemic treatments or insulin treatment.[27] It is feasible that hypoglycaemic treatments may have contributed to the observed discrepancies in the relationship between circulating glucose and ABCG1 expression. For example, insulin decreases human macrophage ABCA1 and ABCG1 gene expression in vitro.[29] Animal studies indicate that complications such as nephropathy independently reduce macrophage ABCA1 and increase cellular cholesterol content.[30] Treatment of nephropathy in this study restored ABCA1-mediated cholesterol efflux in macrophages.[30] The present study has the advantage of having matched controls and patients who were drug naive and free of diabetes related complications.

Skin fibroblasts were used to assess ABCA1 function in our study. Skin fibroblasts have been previously employed for this purpose, where functional consequences of ABCA1 genetic variants have been studied.[8] Our tritiated labelled cholesterol-based technique assessed specific apo-A1-mediated cholesterol efflux from intracellular and membranous sites to the extracellular medium. This was derived from a validated methodology.[19]

It should be noted that our gene expression measurements in leukocytes and cholesterol efflux measurements in fibroblasts were made against very different backgrounds. Leukocyte gene expression is likely to relate to conditions under which the leukocytes were taken at the time of sampling, whereas fibroblasts had undergone prolonged in vitro culture. Despite the prolonged in vitro conditions at the high glucose levels (25mmol/l) present in the culture medium, a relationship was apparent between fibroblast cholesterol efflux and the in vivo glucose concentrations which pertained at the time of sampling. High medium glucose levels have not influenced ABCA1 expression in other studies of leukocytes [31] and the results imply a prolonged metabolic memory on the part of the cells in humans. Longitudinal studies in both type I and type 2 diabetes have demonstrated that intensive glucose lowering leads to fewer cardiovascular disease events many years later, indicating a possible “legacy” effect.[32,33] The mechanisms underlying this prolonged benefit are unknown. The preserved effect of glycaemia, at the time of sampling, on ABCA1 function in fibroblasts in our study may indicate a possible mechanism.

We have shown that ABCA1-mediated cholesterol efflux is positively related to plasma HDL-C concentration, compatible with its key role in HDL formation. This positive relationship between ABCA1 function and blood HDL-C concentrations has previously only been described in humans with known ABCA1 mutations.[34] and was not observed in fibroblasts taken from patients with the metabolic syndrome.[35] It had been thought that increased intravascular HDL remodelling and catabolism were the cause of lower HDL-C concentrations in patients with type 2 diabetes [36] but our observations suggest that reduced HDL synthesis as a result of impaired ABCA1 function may be a factor. The positive relationship between LXRα, ABCG1 and ABCA1 expression testifies to the important role played by LXRα in the regulation of both ABCA1 and ABCG1 in humans. Although unsaturated fatty acids negatively regulate ABCA1 expression in vitro,[12] no relationship was observed with plasma NEFA levels in our study. PPARγ mRNA levels did not significantly relate to ABCA1 expression in blood leukocytes nor to blood glucose levels. The latter could be due to altered transcription factor activity or modification as PPARγ phosphorylation has been shown to influence the glucose lowering effect of the PPARγ agonist, rosiglitazone.[37] ABCG1 has a major role in cholesterol efflux in humans. However, a human disease caused by ABCG1 dysfunction has not been identified and ABCG1 deficiency in animal studies has not consistently shown evidence of increased atherosclerosis. This questions whether ABCG1 plays an equally critical role in preventing atherosclerosis. Unlike ABCA1, ABCG1 does not have a major role in maintaining plasma HDL-C levels.[38]

Genetic variation in ABCA1 can lead to functional decline. This is maximal in Tangier disease and observed to a lesser extent in people with other genetic variants.[39] There have been reports that some variants may occur more commonly in T2DM compared to the background population.[40,41] It is currently unknown whether ABCA1 genetic variation leads to altered gene expression.

It has been proposed that reduced ABCA1 action in humans leads to impaired β-cell function and reduced insulin secretion has been reported in some Tangier patients.[42,43] Both ABCA1 and ABCG1 have been implicated in islet cell function in mice with ABCG1 shown to have a central role in insulin secretion.[44,45] PPARγ agonists may reduce glucose levels in animals through the action of ABCA1 in the β-cell.[44] These data suggest that ABCA1 directly influences glycaemia via its action on β-cell insulin secretion, but other data suggest that it is glucose which modifies ABCA1. Recent animal studies have shown decreased ABCA1 protein and subsequent cellular cholesterol accumulation in macrophages using different mouse models of type 1 diabetes.[13] This has been observed in other animal models.[46] Hyperglycaemia specifically down-regulates ABCA1 and ABCG1 mRNA and protein content in human macrophages in vitro.[21] Similarly, reduction in gene expression has been reported in mouse peritoneal macrophages exposed to high glucose concentrations.[47] In vascular smooth muscle, hyperglycaemia leads to reductions in ABCA1 mRNA and protein through changes in ABCA1 promoter activity, possibly via the p38-mitogen-activated protein kinase (MAPK) pathway.[48] These studies, along with our findings in humans, are compatible with a common glycaemia-mediated suppression of ABCA1. There may, nevertheless be reciprocal interactions between ABCA1 and glycaemia as decompensation in glucose metabolism progresses and this is an area requiring future study.
Our results are the first demonstration of a relationship between glycemia, ABCA1 expression, ABCA1 protein content and cholesterol removal from cells in humans. Collectively, they suggest that T2DM is associated with impaired cellular cholesterol removal via effects on ABCA1 gene expression and function, impairing ABCA1-mediated formation of HDL. In humans information pertaining to RCT is limited. Its importance is highlighted by recent observation that the cholesterol efflux capacity of plasma in man has predicted atherosclerosis and coronary disease. [49] Plasma HDL-C levels accounted for only 40% of the variability in efflux and other processes in cellular cholesterol removal are likely to be important.[49] Insights have historically been discovered from the study of rare genetic disorders of ABCA1, where the degree of transporter dysfunction related to the severity of premature atherosclerosis.[34] There has been substantial therapeutic interest generated by work demonstrating improvements in atherosclerosis burden in cardiac patients after infusions of human recombinant Apo-AI (Milano).[50] Moreover, in men with type 2 diabetes, infusion of HDL resulted in short term improvements in cholesterol removal capacity.[51] Whether augmenting cellular cholesterol removal will effectively prevent future cardiovascular events in susceptible patients remains to be proven.

Supporting Information

Figure S1 The relationship of leukocyte ABCG1 expression to ABCA1 expression and to glycemia. A. Gene expression dCt = δ cycle number (arbitrary units), rho = 0.40, p = 0.005. B. Normalised gene expression versus Hba1c, rho = −0.29, p = 0.05. (TIF)

References

1. Eschwege E, Richard JL, Thibault N, Ducimetiere P, Warnet JM, et al. (1985) Coronary heart disease mortality in relation with diabetes, blood glucose and plasma insulin levels. The Paris Prospective Study, ten years later. Horm Metab Res Suppl 13: 41–46.
2. Khaw KT, Wareham N, Luben R, Bingham S, Oakes S, et al. (2001) Glycated haemoglobin, diabetes, and mortality in men in Norfolk cohort of European Prospective Investigation of Cancer and Nutrition (EPIC-Norfolk). BMJ 322: 15.
3. Yan SF, Ramasamy R, Naka Y, Schmidt AM (2003) Glycation, inflammation, and RAGE: A Scaffold for the Macrophage Complications of Diabetes and Beyond. Circ Res 93: 1159–1169.
4. Krems A (2000) Lipoprotein abnormalities and their consequences for patients with Type 2 diabetes. Diabetes, obesity & metabolism 5: s19.
5. Yamauchi Y, Chang CC, Hayashi M, be-Dohmace S, Reid PC, et al. (2004) Intracellular cholesterol mobilization involved in the ABCA1/apolipoprotein-mediated assembly of high density lipoprotein in fibroblasts. J Lipid Res 45: 1943–1951.
6. Yokoyama S (2005) Assembly of high density lipoprotein by the ABCA1/apolipoprotein pathway. Curr Opin Lipidol 16: 269–279.
7. Gelissen IC, Harris M, Rye KA, Quinn C, Brown AJ, et al. (2006) ABCA1 and ABCG1 Synergize to Mediate Cholesterol Export to ApoA-I. Arterioscler Thromb Vasc Biol 26: 534–540.
8. Lawn RM, Wade DP, Garvin MR, Wang X, Schwartz K, et al. (1999) The Tanger disease gene product ABC1 controls the cellular apolipoprotein-mediated lipid removal pathway. J Clin Invest 104: R25–R31.
9. Aiello RC, Bress D, Bourassa PA, Royer L, Lindsey S, et al. (2002) Increased Atherosclerosis in Hyperlipidemic Mice With Inactivation of ABCA1 in Macrophages. Arteriosclerosis, thrombosis, and vascular biology 22: 630–637.
10. Ormazan J (2000) ABCA1-mediated transport of cellular cholesterol and phospholipids to HDL apolipoproteins. Current Opinion in Lipidology 11: 53–58.
11. Chinetti G, Lestavel S, Bocher V, Remaley AT, Neve B, et al. (2001) PPAR-[α] and PPAR-[γ] activators induce cholesterol removal from human macrophages foam cells through stimulation of the ABCA1 pathway. Nat Med 7: 58–58.
12. Uehara Y, Engel T, Li Z, Goepfert C, Ruit S, et al. (2002) Polyunsaturated Fatty Acids and Acanthocephala Downregulate the Expression of the ATP-Binding Cassette Transporter A1. J Biol Chem 277: 2925–2929.
13. Tang C, Kanter JE, Bournfeldt KE, Lebovic RG, Oram JF (2010) Diabetes reduces the cholesterol exporter ABCA1 in mouse macrophages and kidneys. J Lipid Res 51: 1719–1728.

Figure S2 The relationship of leukocyte Liver X receptor-α (LXRα) gene expression to ABCA1 gene expression and glycemia (Hba1c). A. dCt = δ cycle number (arbitrary units), r = 0.40, p = 0.002. B. Normalised gene expression versus Hba1c, rho = 0.02, p = 0.87. (TIF)

Figure S3 The relationship of leukocyte peroxisome proliferator-activated receptor-γ (PPARγ) gene expression to ABCA1 gene expression and glycemia. A. Normalised gene expression, n = 23, rho = −0.07, p = 0.76. B. Normalised gene expression versus Hba1c, n = 23, rho = 0.31, p = 0.15. (TIF)

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Author Contributions

Conceived and designed the experiments: DCP CA JV DGJ. Performed the experiments: DCP DP VP CP. Analyzed the data: DCP IFG. Contributed reagents/materials/analysis tools: CA SPN. Wrote the paper: DCP CA IFG JV DGJ.
27. Zhou H, Tan KCR, Shiu SWM, Wong Y (2008) Determinants of leukocyte adenosine triphosphate-binding cassette transporter G1 gene expression in type 2 diabetes mellitus. Metabolism 57: 1135–1140.
28. Mauldin JP, Nagelin MH, Wujek AJ, Serivsanan S, Skaflen MD, et al. (2008) Reduced Expression of ATP-Binding Cassette Transporter G1 Increases Cholesterol Accumulation in Macrophages of Patients With Type 2 Diabetes Mellitus. Circulation 117: 2795–2792.
29. Siposbor M, Mouzavi SA, eskild W, Roos N, Berg T (2007) ABCA1, ABCG1 and SR-BI: hormonal regulation in primary rat hepatocytes and human cell lines. BMC Mol Biol 8: 5.
30. Zuo Y, Yanczy P, Castro I, Khan W, Motojima M, et al. (2009) Renal Dysfunction Potentiates Foam Cell Formation by Repressing ABCA1. Arterioscler Thromb Vasc Biol 29: 1277–1282.
31. Senanayake S, Browning LM, Panicker V, Croft KD, Joyce DA, et al. (2007) Monocyte-derived macrophages from men and women with Type 2 diabetes mellitus differ in fatty acid composition compared with non-diabetic controls. Diabetes Research and Clinical Practice 75: 292–300.
32. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group (2005) Intensive Diabetes Treatment and Cardiovascular Disease in Patients with Type 1 Diabetes. N Engl J Med 353: 2643–2653.
33. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA (2008) 10-Year Follow-up of Intensive Glucose Control in Type 2 Diabetes. N Engl J Med 359: 1577–1589.
34. van Dam MJ, de GE, Clee SM, Hovingh GK, Roelants R, et al. (2002) Association between increased arterial-wall thickness and impairment in ABCA1-driven cholesterol efflux: an observational study. Lancet 359: 37–42.
35. Alaczi MI, Marcil M, Blank D, Sherman M, Genest J Jr. (2004) Is the cellular lipid efflux defect? J Clin Endocrinol Metab 89: 2292–2300.
36. Mauldin JP, Nagelin MH, Wojcik AJ, Srinivasan S, Skaflen MD, et al. (2008) Anti-diabetic drugs inhibit obesity-linked phosphorylation of PPAR[g] by Cdk5. Nature 466: 451–456.
37. Choi JH, Banks AS, Estall JL, Kajimura S, Bostrom P, et al. (2010) Expression and Function of ABCA1 in Diabetes Mellitus: 57: 509–513.
38. Kennedy MA, Barrera GC, Nakamura K, Baldan A, Tarr P, et al. (2005) ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. Cell Metab 1: 121–131.
39. Singhara RA, Voscher H, James ER, Chroni A, Coutinho JM, et al. (2006) Specific mutations in ABCA1 have discrete effects on ABCA1 function and lipid phenotypes both in vivo and in vitro. Circ Res 99: 389–397.
40. Daimon M, Kido T, Baba M, Oizumi T, Jimbu Y, et al. (2005) Association of the ABCA1 gene polymorphisms with type 2 DM in a Japanese population. Biochem Biophys Res Commun 329: 205–210.
41. Villarruel-Molina MT, Flores-Dorantes MT, Reina-Campos O, Villalobos-Comparan M, Rodriguez-Cruz M, et al. (2008) Association of the ATP-Binding Cassette Transporter A1 R230C Variant With Early-Onset Type 2 Diabetes in a Mexican Population. Diabetes 57: 509–513.
42. Vergeer M, Brunham LR, Koetsveld J, Kruit JK, Verchere CB, et al. (2010) Carriers of Loss-of-Function Mutations in ABCA1 Display Pancreatic B Cell Dysfunction. Diabetes Care.
43. Koseki M, Matsuyama A, Nakatani K, Inagaki M, Nakaoa H, et al. (2009) Impaired insulin secretion in four Tangier disease patients with ABCA1 mutations. J Atheroscler Thromb 16: 292–296.
44. Brunham LR, Kruit JK, Pepe TD, Timmins JM, Reuwer AQ, et al. (2007) B-cell ABCA1 influences insulin secretion, glucose homeostasis and response to thiazolidinedione treatment. Nat Med 13: 340–347.
45. Sturek JM, Castle JD, Trace AP, Page LC, Castle AM, et al. (2010) An intracellular role for ABCG1-mediated cholesterol transport in the regulated secretory pathway of mouse pancreatic β cells. The journal of clinical investigation 120: 2575–2589.
46. Zhu Y, Wang HJ, Chen L, Fang Q, Yan XW (2007) Study of ATP-binding cassette transporter A1 (ABCA1)-mediated cellular cholesterol efflux in diabetic golden hamsters. J Int Med Res 35: 508–516.
47. Gao F, Yan T, Zhao Y, Yin F, Hu C (2010) A possible mechanism linking hyperglycemia and reduced high-density lipoprotein cholesterol levels in diabetes. J Huazhong Univ Sci Technolog Med Sci 30: 318–321.
48. Yu X, Murao K, Imachi H, Li J, Nishiuchi T, et al. (2010) Hyperglycemia suppresses ABCA1 expression in vascular smooth muscle cells. Horm Metab Res 42: 241–246.
49. Khera AV, Cachel M, de la Llera-Moya M, Rodrigues A, Burke MF, et al. (2011) Cholesterol Efflux Capacity, High-Density Lipoprotein Function, and Atheroecrosis. New England Journal of Medicine 364: 133–143.
50. Patel S, Drew BG, Nakhaa S, Duffy SJ, Murphy AJ, et al. (2009) Reconstituted high-density lipoprotein increases plasma high-density lipoprotein anti-inflammatory properties and cholesterol efflux capacity in patients with type 2 diabetes. J Am Coll Cardiol 53: 962–971.