Published in final edited form as:

Exp Clin Endocrinol Diabetes. 2014 September ; 122(8): 463–468. doi:10.1055/s-0034-1374600.

Regulation of ATP-binding Cassette Transporters and Cholesterol Efflux by Glucose in Primary Human Monocytes and Murine Bone Marrow-derived Macrophages

N. L. Spartano, S. Lamon-Fava, N. R. Matthan, J. Ronxhi, A. S. Greenberg, M. S. Obin, and A. H. Lichtenstein

Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA, United States

Abstract

Purpose—Individuals with type 2 diabetes mellitus are at increased risk of developing atherosclerosis. This may be partially attributable to suppression of macrophage ATP-binding cassette (ABC) transporter mediated cholesterol efflux by sustained elevated blood glucose concentrations. 2 models were used to assess this potential relationship: human monocytes/leukocytes and murine bone marrow-derived macrophages (BMDM).

Methods—10 subjects (4 F/6 M, 50–85 years, BMI 25–35 kg/m²) underwent an oral glucose challenge. Baseline and 1- and 2-h post-challenge ABC-transporter mRNA expression was determined in monocytes, leukocytes and peripheral blood mononuclear cells (PBMC). In a separate study, murine-BMDM were exposed to 5 mmol/L D-glucose (control) or additional 20 mmol/L D- or L-glucose and 25 ug/mL oxidized low density lipoprotein (oxLDL). High density lipoprotein (HDL)-mediated cholesterol efflux and ABC-transporter (ABCA1 and ABCG1) expression were determined.

Results—Baseline ABCA1 and ABCG1 expression was lower (> 50 %) in human monocytes and PBMC than leukocytes ($p < 0.05$). 1 h post-challenge leukocyte ABCA1 and ABCG1 expression increased by 37 % and 30 %, respectively ($p < 0.05$), and began to return to baseline thereafter. There was no significant change in monocyte ABC-transporter expression. In murine BMDM, higher glucose concentrations suppressed HDL-mediated cholesterol efflux (10 %; $p < 0.01$) without significantly affecting ABCA1 and ABCG1 expression. Data demonstrate that leukocytes are not a reliable indicator of monocyte ABC-transporter expression.

Conclusions—Human monocyte ABC-transporter gene expression was unresponsive to a glucose challenge. Correspondingly, in BMDM, hyperglycemia attenuated macrophage cholesterol efflux in the absence of altered ABC-transporter expression, suggesting that hyperglycemia, *per se*, suppresses cholesterol transporter activity. This glucose-related impairment in cholesterol efflux may potentially contribute to diabetes-associated atherosclerosis.

© J. A. Barth Verlag in Georg Thieme Verlag KG Stuttgart

Correspondence: A. H. Lichtenstein, DSc, Cardiovascular Nutrition Laboratory, J.M. USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA, USA 02111, Tel.: + 1/617/556 3127, Fax: + 1/617/556 3103, alice.lichtenstein@tufts.edu.

Conflict of interest: None.

Keywords

atherosclerosis; mononuclear cells; leukocytes; hyperglycemia

Introduction

Individuals with poor glycemic control are at increased risk of developing atherosclerosis [1–3]. In the arterial wall, monocyte-derived macrophages engulf modified low density lipoprotein (LDL), playing a key role in the progression of atherosclerosis [4, 5]. High density lipoprotein (HDL) particles can modify arterial cholesterol accumulation by facilitating reverse cholesterol transport (RCT), the process by which excess cholesterol is removed from macrophages in the arterial wall [5].

Critical to the RCT process are the ATP-binding cassette (ABC)-transporters ABCA1 and ABCG1, and the scavenger receptor (SR)-B1, located on the macrophage membrane [5–7]. Transcription of ABC-transporters is tightly regulated by liverx-receptor (LXR)-alpha (α) [6, 7]. The importance of these transporters in atherosclerosis progression has been observed both in animal models [8–10] and patients with Tangier Disease, the latter carrying mutations in the ABCA1 gene [11–13].

Data from human and animal studies suggest that hyperglycemia may suppress ABC-transporters in macrophages [14–21]. However, the acute effects of elevated blood glucose concentrations have not been examined in this context, *in vivo*. In humans, the concentration of macrophages in circulation is not high enough to harvest a sufficient number to study in isolation. To circumvent this problem, a common approach is to isolate a mixed population of monocytes by plate adherence, a lengthy process that results in differentiation into macrophages, *ex vivo*. Alternatively, leukocytes, peripheral blood mononuclear cells (PBMC) or lymphocytes are often used as a surrogate for monocytes/macrophages in studies designed to evaluate gene and protein expression [20–22]. Leukocytes include a mixture of granulocytes and PBMC (primarily monocytes/macrophages and lymphocytes). Some data suggest that PBMC may not be a good indicator of monocyte ABC-transporter expression [22]. Data are lacking as to whether leukocyte ABC-transporter expression is a good indicator of monocyte expression.

The aims of this work were to assess the reliability of leukocyte ABC-transporter expression to serve as an indicator of monocyte expression and to determine the effect of elevated glucose concentrations on cholesterol efflux in primary monocyte/macrophage models. To address these aims leukocyte and monocyte ABC-transporter expression was characterized, *in vivo*, in response to an acute change in blood glucose concentrations, and ABC-transporter and SR-B1 expression, and macrophage cholesterol efflux was determined, *in vitro*, using murine bone marrow-derived macrophages (BMDM) in response to elevated glucose concentrations.

Materials and Methods

Subjects

10 normoglycemic subjects (4 postmenopausal women and 6 men; age 50–85 years; BMI 25–35 kg/m²) participated in this study. Mean ($\pm$ SD) fasting glucose, insulin, triglycerides (TG), and total-, LDL-, and HDL-cholesterol concentrations were 97 ± 12 mg/dL, 14 ± 3 μ u/mL, 94 ± 71 mg/dL, 189 ± 35 mg/dL, 123 ± 30 mg/dL, 48 ± 10 mg/dL, respectively. Our sample size varied from 4–9 for some outcome measures due to limited sample volumes (see figure legends for details). Exclusion criteria included use of medications known to affect glucose or lipid metabolism. Subjects were asked to refrain from consuming alcohol or engaging in strenuous physical activity 72 h and fast overnight (12 h) prior to the test day. This protocol was approved by the Institutional Review Board at Tufts University. All subjects gave written informed consent.

Oral glucose challenge

A baseline blood sample (time = 0) was collected after an overnight fast. Thereafter, subjects were given an oral glucose challenge, 50 g glucose/50 mL water. Additional blood samples were drawn at 60 and 120 min post-challenge. Plasma glucose and insulin concentrations were measured as previously described [23]. Additionally, blood was collected in PAXgene blood RNA tubes (Qiagen, Valencia, CA) for the analysis of leukocyte gene expression and in BD vacutainer cell preparation tubes (CPT) containing sodium citrate (Becton Dickinson GmbH, Heidelberg, Germany) for analysis of gene expression from PBMC's and monocyte cells, as described below.

Magnetic-activated cell sorting (MACS)

PBMC were separated from other blood cells using the BD vacutainer CPT. Monocytes were isolated from PBMC using Monocyte Isolation Kit II with a magnetic cell sorter (autoMACS pro, Miltenyi Biotec, Bergisch Gladbach, Germany). PBMC were incubated with biotin-conjugated monoclonal antibodies to CD3, CD7, CD16, CD19, CD56, CD123 and glycophrin A, followed by incubation with anti-biotin monoclonal antibodies conjugated to magnetic beads. The autoMACS pro was used to deplete PBMC of magnetically labeled cells, leaving a monocyte-rich fraction. To determine the potential effect of the cell sorter procedure on PBMC gene expression, PBMC from fasting blood samples ($n = 4$) were incubated with the Monocyte Isolation Kit II antibodies conjugated to magnetic beads but were not subjected to cell sorting.

Murine-BMDM cell culture

Bone marrow cells were isolated from the femurs and tibias from 10–12 week old male C57BL/6 mice (Jackson Laboratories, Bar Harbor, ME) by flushing the bone cavity with RPMI medium (Gibco, Life Technologies, Grand Island, NY) as previously described [24]. The harvested cells were washed, plated and differentiated into BMDM by exposing the cells to 100 ng/mL macrophage-colony stimulating factor (eBioscience, San Diego, CA), 20 % low endotoxin fetal bovine serum (FBS) (Gibco, Life Technologies) and 1 % streptomycin/penicillin (Sigma-Aldrich, St. Louis, MO). After 4–5 days, macrophages

adhered to the culture dishes. BMDM differentiation was confirmed by monitoring the expression of the cell surface marker F4/80, preferentially expressed by mature macrophages [24]. Passage of BMDM was achieved by incubation (37 °C, 10 min) with 8 mg/mL lidocaine solution (Sigma-Aldrich). After passage, BMDM were pre-treated for 16 h with 5 mmol/L D-glucose, 25 mmol/L D-glucose or 5 mmol/L D-glucose + 20 mmol/L L-glucose (Sigma-Aldrich) in the presence of 20 % lipoprotein deficient (LD)-FBS. L-glucose was used to control for osmolality. LD-FBS was obtained by ultracentrifugation of FBS at a density of 1.215 g/ml as previously described [25]. After exposure to 25 ug/mL oxidized LDL (oxLDL) (Intracel, Frederick, MD) for 24 h lipid accumulation and mRNA and protein expression were assessed. This protocol was approved by the Tufts University Institution Animal Care and Use Committee.

Quantitative real-time PCR and Western blot analysis

Leukocyte RNA was extracted from whole blood using the PAXgene blood RNA tubes and the PAXgene blood RNA kit (Qiagen). RNA was extracted from human PBMC, monocytes, PBMC depleted of monocytes, and murine BMDM using TRIzol reagent (Ambion, Life Technologies) and Rneasy mini kit (Qiagen). Reverse transcription was performed using the Reverse Transcription Kit (Promega, Madison, WI). Real Time PCR was performed using Qiagen's Quantitect primer assays for human ABCA1, ABCG1, and beta (β)-actin (QT00064869, QT00021035, QT01680476) and murine ABCA1, ABCG1, SR-B1, LXR- α and β -actin (QT00165690, QT00113519, QT00166495, QT00113729, QT01136772).

BMDM protein concentrations were determined by MicroBCA protein assay (Bio-Rad). Proteins were separated by SDS-PAGE (Bio-Rad) under reducing conditions. Quantification by Western blotting was performed using the following primary antibodies: ABCA1 (Abcam, Cambridge, MA), ABCG1 (Santa Cruz Biotechnology, Santa Cruz, CA), SR-B1 (Santa Cruz), LXR- α (Abcam), and β -actin (Sigma-Aldrich). Secondary antibodies were purchased from Santa Cruz Biotechnology.

Assessment of BMDM lipid composition

After lipid extraction [26], cellular TG content was determined using a colorimetric kit (Sigma-Aldrich). Cellular total cholesterol (TC) and free cholesterol (FC) content was determined by gas chromatography using 5- α cholestane as an internal standard [27]. Cholesteryl esters (CE) were calculated as the difference between TC and FC. Cellular lipids (FC, TC, CE and TG) were expressed as μ g/mg cell protein.

Cholesterol efflux assay

After a 16 h incubation with different glucose concentrations BMDM were incubated with 1 uCi/ml [3 H] cholesterol (Perkin Elmer, Waltham, MA) and 25 ug/mL oxLDL for 24 h [28, 29]. Immediately thereafter the cells were equilibrated in RPMI containing 2 mg/mL fatty acid-free bovine serum albumin for 6 h. BMDM were then incubated with either 50 ug/mL HDL (Intracel) or 10 ug/mL apoA1 (Sigma-Aldrich) in serum-free media for 4 h [28]. D- or L-glucose concentrations in the media were maintained constant throughout the experiments. Fraction cholesterol efflux was calculated as the ratio of the radiotracer in the medium to the total (medium + cells), normalized for cellular protein.

Statistical analysis

All data are reported as mean $\pm$ standard deviation (SD). Student's t-test or analysis of variance (ANOVA) was used to test for significant differences between or among groups, respectively. Post-hoc comparisons were performed using Tukey's test (SAS version 9.3, SAS Institute Inc, Cary, NC). A p -value < 0.05 was considered significant.

Results

In vivo, fasting PBMC and monocyte ABC-transporter mRNA is lower than in leukocytes

To assess the reliability of leukocyte and PBMC expression of ABC-transporters as surrogate indicators of monocyte ABCA1 and ABCG1 expression, comparisons were made across these cell types isolated from subjects at time = 0. The expression of ABCA1 and ABCG1 mRNA was significantly lower in monocytes (51 % [$p < 0.05$] and 85 % [$p < 0.0001$], respectively) and PBMC (57 % and 53 % [both $p < 0.01$]) than in total leukocytes (Fig. 1a, b). To rule out the possibility that the antibodies used during the cell sorting protocol changed ABC-transporter mRNA expression, PBMC were incubated with and without the sorting antibodies conjugated to magnetic beads, resulting in no significant effect of treatment.

While subsequent cell sorting had no significant effect on ABCA1 mRNA expression in monocytes or PBMC depleted of monocytes (Fig. 1a), a significantly lower expression of ABCG1 mRNA was observed in monocytes ($p < 0.01$) than in PBMC depleted of monocytes (Fig. 1b). These results indicate that the antibodies conjugated to magnetic beads had no significant effect on ABC transporter expression in PBMC, but there is a differential expression of ABC-transporters in various blood cell types.

Leukocyte ABC-transporter expression increases during an oral glucose challenge

Having established that the isolation procedure did not affect the measures of interest, leukocyte and monocyte ABC-transporter expression was evaluated during a 2-h oral glucose (50 g) challenge. Mean plasma glucose and insulin concentrations (97 ± 12 mg/dL and 14.3 ± 3.3 uU/mL, respectively at baseline) increased by 60 min post-challenge (to 172 ± 34 mg/dL and 74.0 ± 21.6 uU/mL), but fell considerably by 120 min post-challenge (120 ± 27 mg/dL and 44.6 ± 9.5 uU/mL). Average leukocyte ABCA1 and ABCG1 mRNA expression increased by 37 % ($p < 0.01$) and 30 % ($p < 0.05$), respectively, at 60 min post-glucose challenge but did not remain significantly elevated through 120 min (Fig. 2a, b). There was no significant effect of the glucose challenge on monocyte ABCA1 or ABCG1 expression (Fig. 2c, d). These results suggest that monocyte ABC-transporter mRNA expression is not sensitive to an acute increase in plasma glucose concentrations, in vivo. On the basis of these data we assessed the effect of high glucose concentrations on cholesterol efflux and ABC-transporter expression in vitro.

Elevated glucose concentrations suppress HDL-mediated cholesterol efflux in BMDM

Treatment of BMDM with high D- and L-glucose concentrations resulted in lower HDL-mediated cholesterol efflux (10 % [$p < 0.01$] and 5 % [$p < 0.05$], respectively) compared to the low D-glucose concentration (Fig. 3a). There was no significant effect of either D- or L-

glucose concentration on apoA1-mediated cholesterol efflux, although the trend was similar to that of HDL-mediated cholesterol efflux. Since L-glucose is not metabolized by the cells, these results suggest that the effect of high glucose on HDL-mediated cholesterol efflux is not solely dependent on glucose metabolism.

OxLDL exposure, but not glucose, stimulates BMDM CE accumulation

To assess the effect of high glucose on lipid accumulation, BMDM were cultured in 5 mmol/L or 25 mmol/L glucose, followed by exposure to oxLDL. Treatment of BMDM with oxLDL for 24 h resulted in an increase in CE content from undetectable levels to approximately 30 ug/mg protein ($p < 0.0001$) in the absence of significant changes in FC or TG content (Fig. 3c). The increase in CE content was similar across the different glucose concentrations (Fig. 3b). Moreover, glucose concentration in the media did not affect the FC or TG cellular content.

Elevated glucose concentrations do not affect BMDM ABC-transporter expression

Incubation of BMDM with oxLDL resulted in increased ABCA1, ABCG1 and LXR- α mRNA expression and a corresponding increase in ABCA1 protein levels ($p < 0.0001$, Fig. 4a–c). There was no significant effect of oxLDL on SR-B1 mRNA expression or protein levels. These data indicate that exposure to oxLDL in BMDM up-regulates some of the transporters involved in cholesterol efflux. Although high D- and L-glucose decreased HDL-mediated cholesterol efflux (Fig. 3a), there was no significant effect on cholesterol transporter or LXR- α mRNA or protein expression (Fig. 4a–c). In absence of an effect of high glucose concentration on ABC-transporter and SR-B1 transcripts or protein expression, our data suggest that high glucose concentration may impair HDL-mediated cholesterol efflux by attenuating transporter activity.

Discussion

ABC-transporters play an important role in monocyte/macrophage cellular cholesterol balance [5]. Monocyte/macrophage ABC-transporters are involved in the first step of RCT and forestall atherosclerotic lesion progression [5]. Hyperglycemia has been implicated as a potential factor in the disruption of RCT by downregulating monocyte/macrophage ABC-transporter expression and subsequent cholesterol efflux [14–21, 30]. Using BMDM as an experimental model, our results demonstrated that elevated glucose concentrations moderately attenuate cholesterol efflux. In contrast, elevated glucose concentrations had no significant effect on ABC-transporter expression in BMDM or in human monocytes following an oral glucose challenge.

Due to technical challenges in isolating primary monocytes/macrophages, instead, other blood cell fractions and immortalized cell lines have often been used as surrogate cells in studies intended to assess the mechanisms related to cellular cholesterol balance [14, 19, 21, 30]. Since our results indicated that there was no significant change in monocyte ABC-transporter expression following the glucose challenge, the increase in leukocyte ABC-transporter expression may have been coincident with, rather than directly related to, monocyte/macrophage cellular cholesterol balance. Alternatively, the increase in leukocyte

ABC-transporter expression may have been associated with the decrease in plasma free fatty acid concentration which occurs during a glucose challenge [19, 31, 32] and/or an increase in ABC-transporter expression in leukocyte cell types other than PBMC. Hence, it is unlikely that changes in leukocyte ABC-transporter gene expression is a good indicator of changes in monocyte gene expression.

Monocyte ABCA1 and ABCG1 mRNA expression in a commercial preparation of human monocytes has been reported to be higher than in PBMC [22]. In contrast, our results indicate that in non-activated human monocytes ABC-transporter expression is similar to PBMC and lower than in leukocytes. The discrepancy between prior work and our work is likely due to an artifact of the isolation procedures of monocytes from PBMC by plate adherence which has been reported to accelerate conversion to human monocyte-derived macrophages (HMDM) and increases ABC-transporter expression [19]. By design, in the present study, monocytes were isolated by magnetic-activated cell sorting to minimize conversion to macrophages. This method was chosen because it has a shorter sample processing time prior to RNA extraction, hence, maximizes the ability to detect an effect of the glucose challenge on ABC-transporter expression were one present.

Monocyte ABC-transporter expression has not previously been examined in response to a glucose challenge in vivo. However, in vitro studies using a range of 5–25 mmol/L (90 to 450 mg/dL) glucose have documented a suppressive effect on HMDM ABC-transporter expression [19]. In our study, the mean change in plasma glucose response was modest, only 5.4–9.5 mmol/L (97–172 mg/dL), after an oral glucose challenge in normoglycemic subjects. While modest, this response is consistent with the postprandial response to ingestion of a meal. Our results are distinct from previous studies which have reported suppressed HMDM ABC-transporter expression in individuals with type 2 diabetes mellitus (T2DM) compared to normoglycemic individuals [17, 18]. In addition to using a different model (inactivated monocytes), there are factors associated with T2DM, such as insulin resistance, that were not addressed in the current study, which focused on acute elevation of glucose concentrations.

In light of the null effect of the oral glucose challenge on human monocyte ABC-transporter expression, it was of interest to determine the impact of elevated glucose concentrations on ABC-transporter expression in a primary oxLDL-loaded macrophage model, BMDM. Consistent with prior observations, exposing BMDM to oxLDL resulted in intracellular CE accumulation [28], and an increase in ABCA1 and ABCG1 mRNA transcripts and ABCA1 protein expression, possibly mediated by changes in LXR- α activity. However, the suppressive effect of high glucose concentrations on BMDM cholesterol efflux, in the absence of changes in ABC-transporter or SR-B1 protein expression, was unexpected. Although small, the suppressive effect of elevated glucose concentrations on macrophage cholesterol efflux could result in a clinically meaningful cumulative effect if the same mechanism occurs in vivo. We hypothesize that the contrast between our results and prior work [19] may be due to stimulation of BMDM with oxLDL. This stimulation may have overridden potential effects of elevated glucose on cholesterol transporter expression. In the current study, elevated D- and L-glucose concentrations may have directly inhibited ABC-transporter activity. We were unable to determine whether this effect was mediated by

glucose-uptake by the cells, by glycation/glycoxidation of transporters or lipoproteins, which has the potential to suppress cholesterol efflux [33], or by an osmotic effect of elevated glucose concentrations.

Conclusion

This is the first study, to our knowledge, to demonstrate that there is a difference between ABC-transporter expression in human leukocytes compared to monocytes and PBMC. Additionally, our data suggest that elevated glucose concentrations impair HDL-mediated cholesterol efflux and this effect is not mediated by transcript or protein expression but may be mediated by attenuated transporter activity. Future studies should focus on primary monocytes and macrophages to address issues related to elevated glucose concentrations and cholesterol efflux and ABC-transporter expression, rather than using other blood cells or immortalized cell lines as surrogates.

Acknowledgments

Sources of Funding

This work was supported by grants from the NIH: NHLBIT32-HL069772 (NLS) and the USDA agreement No. 58–1950–0–0014. Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture.

The authors would like to thank staff from the following facilities located at the Jean Mayer USDA Human Nutrition Research Center on Aging: the Metabolic Research Unit, Nutrition Evaluation Laboratory and Comparative Biology Unit. We would also like to thank Drs. Alice Dillard-Hirschel and Aleksandra Kristo for their input and assistance with experimental methodology.

References

1. Ryden L, Standl E, Bartnik M, et al. Guidelines on diabetes, pre-diabetes, and cardiovascular diseases: executive summary. The Task Force on Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC) and of the European Association for the Study of Diabetes (EASD). *Eur Heart J*. 2007; 28:88–136. [PubMed: 17220161]
2. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. *Jama*. 2002; 287:2570–2581. [PubMed: 12020339]
3. Park L, Wexler D. Update in diabetes and cardiovascular disease: synthesizing the evidence from recent trials of glycemic control to prevent cardiovascular disease. *Curr Opin Lipidol*. 2010; 21:8–14. [PubMed: 19829110]
4. Williams KJ, Tabas I. The response-to-retention hypothesis of early atherogenesis. *Arterioscler Thromb Vasc Biol*. 1995; 15:551–561. [PubMed: 7749869]
5. Glass CK, Witztum JL. Atherosclerosis. the road ahead. *Cell*. 2001; 104:503–516. [PubMed: 11239408]
6. Jessup W, Gelissen IC, Gaus K, et al. Roles of ATP binding cassette transporters A1 and G1, scavenger receptor BI and membrane lipid domains in cholesterol export from macrophages. *Curr Opin Lipidol*. 2006; 17:247–257. [PubMed: 16680029]
7. Wang X, Rader DJ. Molecular regulation of macrophage reverse cholesterol transport. *Curr Opin Cardiol*. 2007; 22:368–372. [PubMed: 17556891]
8. Aiello RJ, Brees D, Bourassa PA, et al. Increased atherosclerosis in hyperlipidemic mice with inactivation of ABCA1 in macrophages. *Arterioscler Thromb Vasc Biol*. 2002; 22:630–637. [PubMed: 11950702]

9. van Eck M, Bos IS, Kaminski WE, et al. Leukocyte ABCA1 controls susceptibility to atherosclerosis and macrophage recruitment into tissues. *Proc Natl Acad Sci USA*. 2002; 99:6298–6303. [PubMed: 11972062]
10. van Eck M, Singaraja RR, Ye D, et al. Macrophage ATP-binding cassette transporter A1 overexpression inhibits atherosclerotic lesion progression in low-density lipoprotein receptor knockout mice. *Arterioscler Thromb Vasc Biol*. 2006; 26:929–934. [PubMed: 16456089]
11. Bodzioch M, Orso E, Klucken J, et al. The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. *Nat Genet*. 1999; 22:347–351. [PubMed: 10431237]
12. Brooks-Wilson A, Marcil M, Clee SM, et al. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. *Nat Genet*. 1999; 22:336–345. [PubMed: 10431236]
13. Rust S, Rosier M, Funke H, et al. Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. *Nat Genet*. 1999; 22:352–355. [PubMed: 10431238]
14. Passarelli M, Tang C, McDonald TO, et al. Advanced glycation end product precursors impair ABCA1-dependent cholesterol removal from cells. *Diabetes*. 2005; 54:2198–2205. [PubMed: 15983222]
15. Tang C, Kanter JE, Bornfeldt KE, et al. Diabetes reduces the cholesterol exporter ABCA1 in mouse macrophages and kidneys. *J Lipid Res*. 2010; 51:1719–1728. [PubMed: 19965614]
16. Mauldin JP, Srinivasan S, Mulya A, et al. Reduction in ABCG1 in Type 2 diabetic mice increases macrophage foam cell formation. *J Biol Chem*. 2006; 281:21216–21224. [PubMed: 16723355]
17. Mauldin JP, Nagelin MH, Wojcik AJ, et al. Reduced expression of ATP-binding cassette transporter G1 increases cholesterol accumulation in macrophages of patients with type 2 diabetes mellitus. *Circulation*. 2008; 117:2785–2792. [PubMed: 18490524]
18. Zhou H, Tan KC, Shiu SW, et al. Determinants of leukocyte adenosine triphosphate-binding cassette transporter G1 gene expression in type 2 diabetes mellitus. *Metabolism*. 2008; 57:1135–1140. [PubMed: 18640393]
19. Mauerer R, Ebert S, Langmann T. High glucose, unsaturated and saturated fatty acids differentially regulate expression of ATP-binding cassette transporters ABCA1 and ABCG1 in human macrophages. *Exp Mol Med*. 2009; 41:126–132. [PubMed: 19287193]
20. Albrecht C, Simon-Vermot I, Elliott JI, et al. Leukocyte ABCA1 gene expression is associated with fasting glucose concentration in normoglycemic men. *Metabolism*. 2004; 53:17–21. [PubMed: 14681836]
21. Ghanbari-Niaki A, Saghebjo M, Hedayati M. A single session of circuit-resistance exercise effects on human peripheral blood lymphocyte ABCA1 expression and plasma HDL-C level. *Regul Pept*. 2011; 166:42–47. [PubMed: 20727372]
22. DiBlasio-Smith EA, Arai M, Quinet EM, et al. Discovery and implementation of transcriptional biomarkers of synthetic LXR agonists in peripheral blood cells. *J Transl Med*. 2008; 6:1479–5876.
23. Das SK, Gilhooly CH, Golden JK, et al. Long-term effects of 2 energy-restricted diets differing in glycemic load on dietary adherence, body composition, and metabolism in CALERIE: a 1-y randomized controlled trial. *Am J Clin Nutr*. 2007; 85:1023–1030. [PubMed: 17413101]
24. Nguyen MT, Favelyukis S, Nguyen AK, et al. A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. *J Biol Chem*. 2007; 282:35279–35292. [PubMed: 17916553]
25. Terpstra AH. Isolation of serum chylomicrons prior to density gradient ultracentrifugation of other serum lipoprotein classes. *Anal Biochem*. 1985; 150:221–227. [PubMed: 4083481]
26. Schwartz DM, Wolins NE. A simple and rapid method to assay triacylglycerol in cells and tissues. *J Lipid Res*. 2007; 48:2514–2520. [PubMed: 17717377]
27. Matthan NR, Giovanni A, Schaefer EJ, et al. Impact of simvastatin, niacin, and/or antioxidants on cholesterol metabolism in CAD patients with low HDL. *J Lipid Res*. 2003; 44:800–806. [PubMed: 12562840]
28. Meyer JM, Ji A, Cai L, et al. High-capacity selective uptake of cholesteryl ester from native LDL during macrophage foam cell formation. *J Lipid Res*. 2012; 53:2081–2091. [PubMed: 22833685]
29. Wang Y, Oram JF. Unsaturated fatty acids phosphorylate and destabilize ABCA1 through a protein kinase C delta pathway. *J Lipid Res*. 2007; 48:1062–1068. [PubMed: 17325386]

30. Zhou H, Tan KC, Shiu SW, et al. Cellular cholesterol efflux to serum is impaired in diabetic nephropathy. *Diabetes Metab Res Rev*. 2008; 24:617–623. [PubMed: 18802933]
31. Zheng Z, Luo Y, McMaster GK. Sensitive and quantitative measurement of gene expression directly from a small amount of whole blood. *Clin Chem*. 2006; 52:1294–1302. [PubMed: 16709626]
32. Feezor RJ, Baker HV, Mindrinos M, et al. Whole blood and leukocyte RNA isolation for gene expression analyses. *Physiol Genomics*. 2004; 19:247–254. [PubMed: 15548831]
33. Uehara Y, Miura S, von Eckardstein A, et al. Unsaturated fatty acids suppress the expression of the ATP-binding cassette transporter G1 (ABCG1) and ABCA1 genes via an LXR/RXR responsive element. *Atherosclerosis*. 2007; 191:11–21. [PubMed: 16730733]
34. Punyadeera C, van der Merwe MT, Crowther NJ, et al. Weight-related differences in glucose metabolism and free fatty acid production in two South African population groups. *Int J Obes Relat Metab Disord*. 2001; 25:1196–1205. [PubMed: 11477505]
35. Machado-Lima A, Iborra RT, Pinto RS, et al. Advanced glycated albumin isolated from poorly controlled type 1 diabetes mellitus patients alters macrophage gene expression impairing ABCA-1-mediated reverse cholesterol transport. *Diabetes Metab Res Rev*. 2013; 29:66–76. [PubMed: 23015358]

Fig. 1.

Percent differences of mRNA expression in cells isolated from subjects in the fasting state. **a** ABCA1 and **b** ABCG1 mRNA expression was evaluated by real-time PCR. β -actin was used as the standard housekeeping gene. The abundance of mRNA in leukocytes was defined as 100 %, and the amounts of ABCA1 and ABCG1 mRNA from each cell fraction were then expressed as percentages of this control value. Values represent mean $\pm$ SD ($n = 4$). * $p < 0.05$, † $p < 0.01$, § $p < 0.0001$ compared to leukocytes. # $p < 0.01$ compared to monocytes.

Fig. 2.

Effect of an oral glucose challenge on ABC transporter expression in leukocytes and monocytes. **a** Leukocyte ABCA1 and **b** ABCG1 and **c** monocyte ABCA1 and **d** ABCG1 mRNA expression was determined by real-time PCR. β -actin was used as a standard housekeeping gene. Values represent mean $\pm$ SD; leukocytes ($n = 9$) or monocytes ($n = 7$). * $p < 0.05$, † $p < 0.01$ compared to fasting (time = 0).

Fig. 3.

Effect of elevated glucose (glu) on lipid accumulation and cholesterol efflux in BMDM. **a** OxLDL- and [³H] cholesterol-loaded BMDM were stimulated with 50 ug/mL HDL or 10 ug/mL apoA1 for 4 h. Fractional cholesterol efflux was estimated from the ratio of the radiotracer in the medium to the total (medium + cells) and normalized for cellular protein. **b** and **c** Lipid was first extracted from cells. Triglyceride (TG) was determined by colorimetric TG Accumulation Kit to analyze the effect of glucose concentrations **b** and oxLDL **c** on lipid accumulation. Free cholesterol (FC) and cholesteryl esters (CE) were determined using gas chromatography. Values are representative of 3 independent experiments in triplicate. * $p < 0.05$, [†] $p < 0.01$ compared to 5 mmol/L D-glu + oxLDL treated BMDM. § $p < 0.0001$ compared to 5 mmol/L D-glu treated BMDM.

Fig. 4.

Effect of elevated glucose (glu) and oxLDL on ABCA1, ABCG1, S-B1 and LXR- α mRNA and protein expression in BMDM. **a** ABCA1, ABCG1, SR-B1 and LXR- α mRNA expression was determined by real-time PCR. β -actin was used as a standard housekeeping gene. **b** ABCA1, ABCG1, SR-B1, LXR- α and β -actin protein expression was determined by Western blot analysis of BMDM cell lysates. β -actin was used to control equal loading. **c** A representative Western blot used for evaluation of protein expression **b**. Values are representative of 3 independent experiments in triplicate. ‡ $p < 0.001$, § $p < 0.0001$ compared to 5 mmol/L D-glu treated BMDM.