

# Replacement of dietary saturated FAs by PUFAs in diet and reverse cholesterol transport

I. Kralova Lesna,<sup>1</sup> P. Suchanek, J. Kovar, P. Stavek, and R. Poledne

Institute for Clinical and Experimental Medicine, Center for Cardiovascular Research, Prague, Czech Republic

**Abstract** Dietary intervention is the first and usually successful approach in the treatment of high LDL cholesterol (LDL-C) concentration, but it is frequently accompanied by a decrease in HDL concentration. We studied 14 male volunteers on two different diets, high saturated fatty acid (SFA) and high PUFA, in a crossover design to test whether a decrease in HDL can affect reverse cholesterol transport from relabeled macrophages. A significant decrease of LDL-C (in mmol/l) after a PUFA diet compared with an SFA diet from  $3.15 \pm 0.65$  to  $2.80 \pm 0.56$  ( $P < 0.01$ ) was accompanied by a significant decrease of HDL cholesterol (HDL-C) (in mmol/l) from  $1.21 \pm 0.30$  to  $1.10 \pm 0.32$  ( $P < 0.05$ ). These changes did not affect cholesterol efflux (CHE) from macrophages ( $9.74 \pm 1.46\%$  vs.  $9.53 \pm 1.41\%$ ). There was no correlation between individual changes of HDL-C and changes of CHE. It is concluded that the decrease of HDL-C after successful dietary intervention of LDL-C is not accompanied by a decrease of CHE.—Kralova Lesna, I., P. Suchanek, J. Kovar, P. Stavek, and R. Poledne. Replacement of dietary saturated FAs by PUFAs in diet and reverse cholesterol transport. *J. Lipid Res.* 2008. 49: 2414–2418.

**Supplementary key words** fatty acids • cholesterol efflux • macrophages

Coronary heart disease (CHD) due to atherosclerosis of the coronary arteries is the main cause of mortality in the Western world (1). One of the typical phenotypes associated with increased risk of CHD includes increased LDL cholesterol (LDL-C) and reduced concentrations of HDL. Several epidemiological and physiological data indicate that a change in lifestyle (diet, physical training) can significantly improve lipoprotein profile and prognosis of CHD (2, 3). Meta-analysis of 60 controlled trials (4), focused on the effects of dietary FAs, clearly demonstrated a significant decrease in LDL concentration when saturated fatty acids (SFAs) in a diet were replaced with unsaturated FAs, and this decrease was accompanied by a significant decrease in HDL concentration. It is not clear whether such a decrease of HDL cholesterol (HDL-C) con-

centration compromises the otherwise atheroprotective effect of such a diet change.

The importance of low concentrations of HDL as risk factor for cardiovascular diseases was initially demonstrated in Miller et al. (5), and this finding has been repeatedly confirmed (6, 7).

HDL represents a large family of particles differing in size, shape, and composition. Among several protective actions of HDL [protection of endothelial function, and anti-oxidative, anti-inflammatory, and anti-aggregative effects (8–10)], the capacity of HDL to accept cellular lipids and reverse cholesterol transport (RCT) are thought to be the most important. RCT is the key pathway for efflux of cholesterol molecules to extracellular acceptors and their transport to the liver to be metabolized to bile acids. Despite the fact that RCT from macrophages contributes only a little to the whole centripetal transport of cholesterol, this pathway is crucial (11), inasmuch as accumulation of esterified cholesterol in these cells is one of the first features of atherosclerosis.

RCT is a complex process involving several steps. First, unesterified cholesterol is carried out to the plasma membrane and then is taken up into extracellular space. Although some nonspecific processes may be involved (12), the removal of cellular cholesterol is realized almost entirely through interaction between cellular receptors such as ABCA1, adenosine triphosphate binding cassette transporter G1 (ABCG1), and scavenger receptor class B type 1 (SR-BI) and plasma acceptors (pre- $\beta$ -HDL and mature spherical HDL) (13, 14). It has been proven (15) that some intracellular cholesterol leaves macrophages together with endogenously synthesized apolipoprotein E (apoE). The free cholesterol in HDL is then esterified through LCAT action, and mature spherical HDL particles are formed and remodeled in plasma by the effect of

Abbreviations: ABCG1, adenosine triphosphate binding cassette transporter G1; apo, apolipoprotein; CHD, coronary heart disease; FAFA, fatty acid-free bovine serum albumin; MUFA, monounsaturated fatty acid; NEFA, non-esterified fatty acid; RCT, reverse cholesterol transport; SFA, saturated fatty acid; SR-BI, scavenger receptor class B type 1.

<sup>1</sup>To whom correspondence should be addressed.  
e-mail: ivka@ikem.cz

This work was financially supported by Internal Grant Agency of the Ministry of Health of the Czech Republic, Project NR 8486-4.

Manuscript received 22 May 2008 and in revised form 7 July 2008.

Published, JLR Papers in Press, July 9, 2008.  
DOI 10.1194/jlr.M800271-JLR200

other enzymes, cholesteryl ester transfer protein, phospholipid transfer protein, and endothelial and hepatic lipases.

Owing to the complexity of the RCT process, a detailed study of the particular steps of RCT may not be enough to fully understand RCT. However, to overcome such complexity, methods that use diluted serum or plasma as an acceptor of cholesterol from cultivated cells may be used. Fibroblasts (16, 17), hepatocyte cell lines (18, 19), and macrophages (20, 21) were used mainly as the cell model for cholesterol efflux (CHE) measurements, and the methods of the measurements of CHE from cultivated cells were recently described in detail (22).

Therefore, we decided to use such a method of CHE measurement to determine whether the decrease of HDL-C in a diet enriched with polyunsaturated fat is indeed detrimental with respect to RCT. The CHE was measured in young, healthy men in whom cholesterol, LDL-C, and HDL-C were lowered by replacement of SFAs by PUFAs in the diet.

## MATERIALS AND METHODS

### Subjects

Fourteen healthy Caucasian male volunteers participated in the study. Inclusion criteria were self-motivation to participate in the study, age between 18 and 55 years, body mass index  $<30 \text{ kg}\cdot\text{m}^{-2}$  and normal concentration of lipoproteins.

Exclusion criteria included recent adherence to any diet restriction before the study, use of any medications that could affect study outcomes, and diabetes mellitus and/or any other major illness as assessed by medical history, physical examination, and laboratory screening.

None of the subjects used any medication 1 month before and during the study and none of the subjects had an extremely low or high level of physical activity. The design of the study was approved by the Ethics Committee of the Institute for Clinical and Experimental Medicine. Written informed consent was obtained from all study subjects before participating.

### Diets and lifestyle

The study had a randomized crossover design. Two 4 week diet interventions were carried out in succession without a wash-out period. A diet high in saturated fatty acids (SFA diet) or a diet high in polyunsaturated fatty acids (PUFA diet) was provided to participants. The crossover design of the study was used to minimize all confounding effects. Both diets were isocaloric (12,500 kJ/day), providing 45% of energy from carbohydrates, 15% from protein, and 40% from fats. The diets differed considerably in their FA composition. The SFA diet contained 52% SFAs, 34% monounsaturated fatty acids (MUFAs), and 14% PUFAs. The PUFA diet contained 26% SFAs, 33% MUFAs, and 41% PUFAs.

The diet composition was determined by a registered dietician, and the food was prepared at the Institute facility. The participants were asked not to consume any other food during the study, but they were allowed to adjust their food intake to feel comfortable (individualized size portions were balanced every week). All meals were provided to participants (breakfast, a mid-morning snack, lunch, an afternoon snack, and dinner). Diet adherence was monitored by dietary records and repeated sessions with a dietician. Subjects were asked to maintain their usual

physical activities and lifestyles and to record any event that could affect the outcome of the study, particularly illness. Consumption of alcohol was not prohibited up to maximal daily dose of 20 g. We did not follow the use of tobacco during the study. Body weight and waist circumference were monitored throughout the study.

### Biochemical data

Blood samples were obtained by venous puncture and drawn into vacutainer tubes after a 12 h overnight fast at the beginning of the study and at the end of each dietary period. Serum samples for lipid analysis were allowed to coagulate at room temperature, whereas samples for CHE measurements were placed immediately in ice water and allowed to coagulate. Blood samples were centrifuged at 3,700 rpm at 4°C for 10 min. Serum was separated, portioned, and frozen in duplicate and stored at  $-80^{\circ}\text{C}$  until analysis at the end of the study. Plasma triglyceride concentrations, and total cholesterol and LDL-C concentrations were determined enzymatically by commercial kits (Roche Diagnostics). HDL-C concentration was measured after phosphotungstate precipitation of apoB-containing lipoproteins. Non-esterified fatty acid (NEFA) concentrations were measured by an enzymatic test (Wako Chemicals GmbH, Neuss, Germany), and apoA-I and apoB concentrations by immunoturbidimetric assay (Orion Diagnostica, Espoo, Finland). All lipoprotein analysis methods in the laboratory are under the permanent control of the Centers for Disease Control, Atlanta, GA. LDL-C concentration was also calculated from Friedewald's formula.

The distribution of HDL subfractions was determined at the end of each dietary period after separation of lipoproteins ( $d < 1.21 \text{ g/ml}$ ) by ultracentrifugation using nondenaturing PAGE as described by others (23). Both samples from each subject were run on the same gel to eliminate interassay variation.

### Anthropometric data

Anthropometric data (weight and waist circumference) were determined at the beginning of the study and at the end of each dietary regimen.

### Cell culture and efflux measurements

RCT was measured using *in vitro* in cells pre-labeled in the medium containing labeled cholesterol. Human serum from each subject was used as acceptor of cholesterol released from cells for determination of CHE. THP-1 human monocytes (human monocytic leukemia cells, ECACC 88087201) were maintained in RPMI 1640 medium containing 10% FBS, 2 mM glutamine, and 1% penicillin/streptomycin (PAA Laboratories) at 37°C, 5%  $\text{CO}_2$ .

THP-1 monocytes were seeded into 24-well plates at a density of approximately  $2 \times 10^5$  cells/ml in the presence of phorbol 12-myristate 13-acetate (100 ng/ml) (Sigma-Aldrich) and incubated for 72 h to induce differentiation into macrophages. Before each of the following steps, the cells were washed with PBS (PAA Laboratories), containing 0.1% fatty acid-free bovine serum albumin (FAFA) (PAA Laboratories). THP-1 macrophage cells were labeled during a 48 h incubation in a medium containing [ $^{14}\text{C}$ ]cholesterol (specific activity 0.2  $\mu\text{Ci/ml}$ ) (PerkinElmer Life Sciences, Inc.).

The incubation medium was labeled with [ $^{14}\text{C}$ ]cholesterol in ethanol. The final ratio of ethanol to medium was 1:400. Ethanol solution was slowly added during continuous shaking of the media. The cells were then equilibrated overnight in serum-free RPMI medium containing 0.1% FAFA. To measure CHE, cells were incubated for 240 min in RPMI medium containing 5% serum of study subjects. Each serum aliquot was thawed only

once just prior to use. Our previous unpublished data and other findings (18) showed that one cycle of freezing and thawing did not influence the ability of the serum to stimulate cholesterol release from the cells. Medium containing only FAFA (0.15%) (without tested sera) was used as a control. Efflux of cholesterol was measured between 15 and 240 min because the time curve of efflux of label was linear at this time interval and because the washout of labeled cholesterol off the cell surface was completed within 15 min. Aliquots of efflux media were collected at 15 min, and the efflux phase was ended at 240 min. Media were then centrifuged at 450 *g* at 4°C for 5 min to remove any floating cells, and then supernatants were mixed with scintillation liquid (Rotiszint® 11, Roth, Germany) for liquid scintillation counting. Medium-free cell monolayers were washed twice with ice-cold PBS containing 0.1% FAFA and once with ice-cold PBS. Cells were frozen for at least 1 h and then defrosted and lysed in isopropanol-hexane (2:3) for 1 h. Aliquots of the lysates were centrifuged at 440 *g* and evaporated in the scintillation vials, and then scintillation liquid was added for liquid scintillation counting. Serum samples from each subject were run in the same assay to eliminate interassay variation. The coefficient of variation for the CHE assay was 9.95%.

Percent CHE was expressed as radioactivity (cpm) in the efflux media divided by total radioactivity of the sample (media plus cell) and multiplied by 100. Each serum sample was analyzed in triplicate. Absolute differences in the triplicates of the present experiment were  $\pm 1.2\%$  (SD 0.52).

The measured values of efflux were not corrected to efflux to albumin alone [4.37 (SD 0.28)] and they may include the efflux of labeled cholesterol carried with endogenously synthesized apoE. These parameters should not vary within wells.

### Statistical analysis

All results are expressed as mean  $\pm$  SD. The differences between SFA and PUFA dietary periods were evaluated using a paired *t*-test. The relationship between CHE and lipoprotein parameters and changes of CHE and changes of lipoprotein parameters were analyzed by simple linear regression.

## RESULTS

No significant changes of body weight and waist circumference were documented during either dietary regimen.

As expected, the results of subjects on the PUFA diet showed 9.4% lower concentrations of total cholesterol,

9.3% lower LDL-C, and 9.1% lower HDL-C when compared with those of subjects on the SFA diet (Table 1). A similar trend could be observed for apoB and apoA-I concentrations, but the differences were not statistically significant in spite of a 9.1% decrease of apoA-I. Concentrations of triglycerides and NEFAs, as well as glycemia, were not affected by the diet change.

Importantly, no change in HDL subfraction distribution was observed using SDS-PAGE distribution between both dietary periods; there were neither significant changes in the proportional areas under the curve corresponding to particular HDL subfractions (HDL<sub>2a</sub>, HDL<sub>2b</sub>, HDL<sub>3a</sub>, HDL<sub>3b</sub>, and HDL<sub>3c</sub>) nor significant shifts in position of peaks of HDL distribution patterns (data not shown).

CHE measured using individual serum samples of study subjects at the end of both dietary periods was not different and was comparable to that at baseline (Table 1;  $9.74 \pm 1.46\%$  at the end of the SFA diet and  $9.53 \pm 1.41\%$  at the end of the PUFA diet, with no significant difference). These results were not affected even after adjustment to HDL-C and/or apoA-I concentrations.

No correlation was found between CHE and lipids and lipoprotein concentrations on both diets. Importantly, there was also no correlation between change of CHE and change of HDL-C and apoA-I between diets (Fig. 1).

## DISCUSSION

It could be summarized that the replacement of saturated fat in the diet with polyunsaturated fat does not affect the CHE rate in spite of a significant effect on the HDL-C concentration.

The replacement of a saturated fat diet with a polyunsaturated fat diet and a decrease of alimentary cholesterol intake is an integral part of dietary therapy for hyperlipoproteinemias, but the decrease in LDL is accompanied by a negative change in atheroprotective HDL-C (24–26). The purpose of this study was to induce these changes and to measure coincident changes in CHE.

As expected, the consumption of a PUFA diet resulted in a substantially lower concentration of total cholesterol,

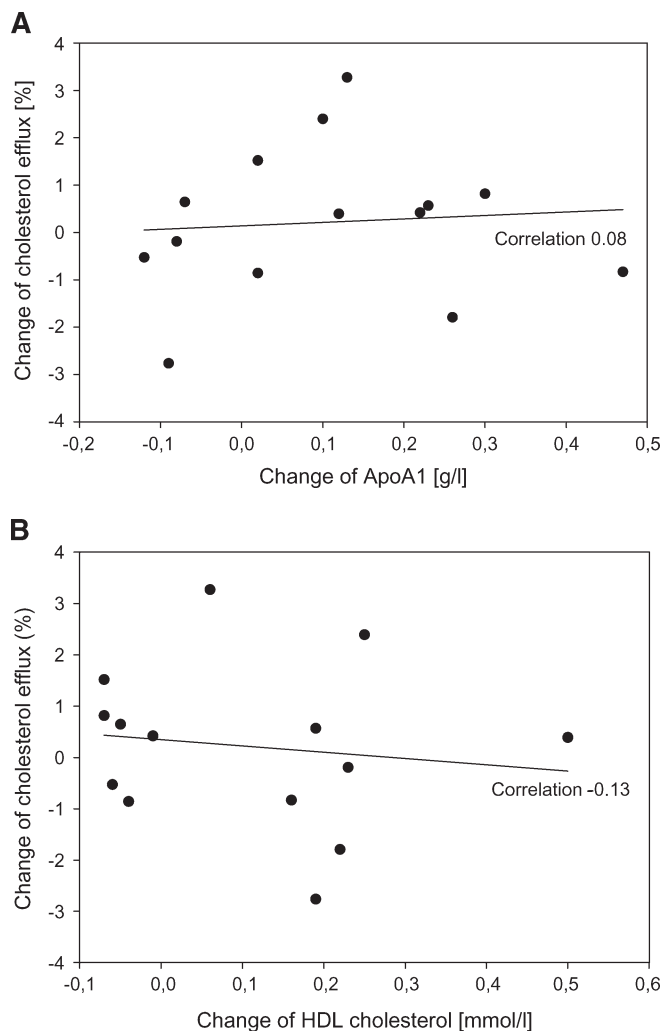
TABLE 1. Concentration of lipids, lipoproteins, and glucose, and the rate of CHE to serum in 14 healthy men at baseline, after 4 weeks of a high SFA diet, and after 4 weeks on a high PUFA diet

	Baseline	SFA diet	PUFA diet
Total cholesterol (mmol/l)	4.82 (0.86)	5.13 (0.85)	4.65 (0.70) <sup>b</sup>
Triglycerides (mmol/l)	1.35 (0.7)	1.69 (0.84)	1.64 (0.71)
LDL cholesterol (mmol/l)	3.24 (0.87)	3.33 (0.69)	3.02 (0.55) <sup>b</sup>
LDL cholesterol calc. (mmol/l)	3.01 (0.82)	3.15 (0.65)	2.80 (0.56) <sup>b</sup>
HDL cholesterol (mmol/l)	1.19 (0.40)	1.21 (0.30)	1.10 (0.32) <sup>a</sup>
ApoB (g/l)	0.87 (0.33)	0.93 (0.24)	0.90 (0.18)
ApoA-I (g/l)	1.29 (0.15)	1.34 (0.16)	1.25 (0.19)
NEFA (mmol/l)	0.26 (0.18)	0.40 (0.11)	0.43 (0.21)
Glucose (mmol/l)	5.35 (0.43)	5.59 (0.58)	5.49 (0.53)
CHE (%)	10.02 (1.44)	9.74 (1.46)	9.53 (1.41)

Apo, apolipoprotein; CHE, cholesterol efflux; NEFA, non-esterified fatty acid; SFA, saturated fatty acid. Data are given as mean  $\pm$  SD.

<sup>a</sup> *P* < 0.05 (SFA vs. PUFA diet, paired *t*-test)

<sup>b</sup> *P* < 0.01 (SFA vs. PUFA diet, paired *t*-test)



**Fig. 1.** Relationship between the change of cholesterol efflux after 4 weeks on a high saturated fat diet and after 4 weeks on a high PUFA diet. A: Change in apolipoprotein A-I (apoA-I) concentration. B: Change in HDL cholesterol. There were no statistically significant correlations between the variables.

LDL-C, and HDL-C, similar to the results of other studies (24–26). The differences in apoB and apoA-I did not reach statistical significance.

Importantly, although the replacement of SFA by PUFA resulted in a change of HDL-C, it had no effect on the rate of CHE from macrophages labeled with [ $^{14}$ C]cholesterol to sera from subjects in the study. To the best of our knowledge, our study is the first to demonstrate that replacement of SFA by PUFA in humans does not influence CHE to serum from the macrophages. In agreement with our findings, Sola et al. (27) did not observe any effect of a PUFA diet compared with an SFA diet but observed the increased capacity of CHE from human fibroblasts on a MUFA diet. However, the same group (28) did not find any difference in RTC from macrophages after a linoleic acid-rich diet and another diet enriched in oleic acid using HDL3 as acceptors. Similar to our findings, Montoya et al.

(24) did not find any effect of a diet enriched with n-6 PUFA on CHE, but did find a positive effect of an n-3 PUFA-rich diet on CHE only. It is important to note that a different cell model, rat hepatoma cells, was used in this study.

Other studies have been carried out to analyze the relationship between HDL-C and CHE after specific lifestyle interventions. Olchawa et al. (20) found higher HDL-C and apoA-I and correspondingly higher efflux of cholesterol in trained subjects and suggested that higher RCT in trained subjects was probably caused by a higher number of HDL particles rather than increased ability of HDL particles to promote CHE from mouse macrophage cells. Similarly, Brites et al. (29) found a correlation between higher HDL level and higher CHE in trained subjects. Van der Gaag et al. (30) and Beulens et al. (19) described a positive effect of chronic use of low doses of alcohol on RCT. However, other authors (31) have found that CHE from macrophages to plasma is impaired in human alcoholics.

It can be objected that results of CHE measurement may be affected by the method used. We decided to use modified macrophages because they play a crucial role in the pathogenesis of atherosclerosis. This cell model has been used frequently in similar studies (32–34), and in addition, the presence of receptors involved in RCT (ABCA1, ABCG1, and SR-BI) was proved in these cells (35, 36). Both whole diluted sera and isolated lipoproteins have been used in studies of CHE as acceptors of cellular cholesterol. We preferred the use of the whole serum because it avoids the pitfalls of changes to HDLs or their subfractions during isolation of subfractions. Although the use of whole serum does not allow discrimination between relative roles of the different pathways and receptors involved in CHE, it is likely to be a best approximation of an *in vivo* situation.

The mechanism of HDL decrease on a PUFA diet has not yet been well characterized. Based on our results, it does not seem likely that the ability of HDL to remove cholesterol from cells is significantly affected. It was shown recently by Uehara et al. (35) that PUFA can lower ABCA1 and ABCG1 gene expression in macrophages. It can therefore be speculated that even if the ability of HDL to remove cellular cholesterol is not affected, the lower activity of cellular receptors on a PUFA diet can result in a decrease of HDL-C concentration.

The measurement of concentration of HDL seems to be only a very rough indicator of the complex process of RCT because no correlation of individual efflux data changes to either HDL or apoA-I concentration changes was found. This suggests that the concentration of HDL particles does not entirely correspond to the kinetics of RCT. A detailed understanding of HDL kinetics could help in assessing a change in cardiovascular risk after different lifestyle and/or medical interventions.

It can be concluded that the decrease of HDL-C resulting from replacement of SFA by PUFA in the diet does not affect the rate of CHE and does not seem to be detrimental to patients. **Fig. 1**

## REFERENCES

- Hennekens, C. H. 1998. Increasing burden of cardiovascular disease: current knowledge and future directions for research on risk factors. *Circulation*. **97**: 1095–1102.
- Aldana, S. G., W. R. Whitmer, R. Greenlaw, A. L. Avins, A. Salberg, M. Barnhurst, G. Fellingham, and L. Lipsenthal. 2003. Cardiovascular risk reductions associated with aggressive lifestyle modification and cardiac rehabilitation. *Heart Lung*. **32**: 374–382.
- Koertge, J., G. Weidner, M. Elliott-Eller, L. Scherwitz, T. A. Merritt-Worden, R. Marlin, L. Lipsenthal, M. Guarneri, R. Finkel, D. E. Saunders, Jr., et al. 2003. Improvement in medical risk factors and quality of life in women and men with coronary artery disease in the Multicenter Lifestyle Demonstration Project. *Am. J. Cardiol*. **91**: 1316–1322.
- Mensink, R. P., P. L. Zock, A. D. Kester, and M. B. Katan. 2003. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am. J. Clin. Nutr*. **77**: 1146–1155.
- Miller, N. E., D. S. Thelle, O. H. Forde, and O. D. Mjos. 1977. The Tromso heart-study. High-density lipoprotein and coronary heart-disease: a prospective case-control study. *Lancet*. **1**: 965–968.
- Genest, J. J., J. R. McNamara, D. N. Salem, and E. J. Schaefer. 1991. Prevalence of risk factors in men with premature coronary artery disease. *Am. J. Cardiol*. **67**: 1185–1189.
- Franceschini, G. 2001. Epidemiologic evidence for high-density lipoprotein cholesterol as a risk factor for coronary artery disease. *Am. J. Cardiol*. **88**: 9N–13N.
- Deakin, S. P., and R. W. James. 2004. Genetic and environmental factors modulating serum concentrations and activities of the anti-oxidant enzyme paraoxonase-1. *Clin. Sci. (Lond.)*. **107**: 435–447.
- Wadham, C., N. Albanese, J. Roberts, L. Wang, C. J. Bagley, J. R. Gamble, K. A. Rye, P. J. Barter, M. A. Vadas, and P. Xia. 2004. High-density lipoproteins neutralize C-reactive protein proinflammatory activity. *Circulation*. **109**: 2116–2122.
- von Eckardstein, A., M. Hersberger, and L. Rohrer. 2005. Current understanding of the metabolism and biological actions of HDL. *Curr. Opin. Clin. Nutr. Metab. Care*. **8**: 147–152.
- Aiello, R. J., D. Brees, P. A. Bourassa, L. Royer, S. Lindsey, T. Coskran, M. Haghpassand, and O. L. Francone. 2002. Increased atherosclerosis in hyperlipidemic mice with inactivation of ABCA1 in macrophages. *Arterioscler. Thromb. Vasc. Biol*. **22**: 630–637.
- Rothblat, G. H., and M. C. Phillips. 1982. Mechanism of cholesterol efflux from cells. Effects of acceptor structure and concentration. *J. Biol. Chem*. **257**: 4775–4782.
- Jessup, W., I. C. Gelissen, K. Gaus, and L. Kritharides. 2006. Roles of ATP binding cassette transporters A1 and G1, scavenger receptor BI and membrane lipid domains in cholesterol export from macrophages. *Curr. Opin. Lipidol*. **17**: 247–257.
- Wang, N., D. Lan, and W. Chen. 2004. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. *Proc. Natl. Acad. Sci. USA*. **101**: 9774–9779.
- Lin, C. Y., H. Duan, and T. Mazzone. 1999. Apolipoprotein E-dependent cholesterol efflux from macrophages: kinetic study and divergent mechanisms for endogenous versus exogenous apolipoprotein E. *J. Lipid Res*. **40**: 1618–1627.
- Marcil, M., L. Yu, L. Krimbou, B. Boucher, J. F. Oram, J. S. Cohn, and J. Genest, Jr. 1999. Cellular cholesterol transport and efflux in fibroblasts are abnormal in subjects with familial HDL deficiency. *Arterioscler. Thromb. Vasc. Biol*. **19**: 159–169.
- Haidar, B., S. Mott, B. Boucher, C. Y. Lee, M. Marcil, and J. Genest, Jr. 2001. Cellular cholesterol efflux is modulated by phospholipid-derived signaling molecules in familial HDL deficiency/Tangier disease fibroblasts. *J. Lipid Res*. **42**: 249–257.
- de la Llera Moya, M., V. Atger, J. L. Paul, N. Fournier, N. Moatti, P. Giral, K. E. Friday, and G. Rothblat. 1994. A cell culture system for screening human serum for ability to promote cellular cholesterol efflux. Relations between serum components and efflux, esterification, and transfer. *Arterioscler. Thromb*. **14**: 1056–1065.
- Beulens, J. W., A. Sierksma, A. van Tol, N. Fournier, T. van Gent, J. L. Paul, and H. F. Hendriks. 2004. Moderate alcohol consumption increases cholesterol efflux mediated by ABCA1. *J. Lipid Res*. **45**: 1716–1723.
- Olchawa, B., B. A. Kingwell, A. Hoang, L. Schneider, O. Miyazaki, P. Nestel, and D. Sviridov. 2004. Physical fitness and reverse cholesterol transport. *Arterioscler. Thromb. Vasc. Biol*. **24**: 1087–1091.
- Kiss, R. S., J. Maric, and Y. L. Maric. 2005. Lipid efflux in human and mouse macrophagic cells: evidence for differential regulation of phospholipid and cholesterol efflux. *J. Lipid Res*. **46**: 1877–1887.
- Waddington, E. I., E. Boadu, and G. A. Francis. 2005. Cholesterol and phospholipid efflux from cultured cells. *Methods*. **36**: 196–206.
- Nichols, A. V., R. M. Krauss, and T. A. Musliner. 1986. Nondenaturing polyacrylamide gradient gel electrophoresis. *Methods Enzymol*. **128**: 417–431.
- Montoya, M. T., A. Porres, S. Serrano, J. C. Fruchart, P. Mata, J. A. Gerique, and G.R. Castro. 2002. Fatty acid saturation of the diet and plasma lipid concentrations, lipoprotein particle concentrations, and cholesterol efflux capacity. *Am. J. Clin. Nutr*. **75**: 484–491.
- Hodson, L., C. M. Skeaff, and W. A. Chisholm. 2001. The effect of replacing dietary saturated fat with polyunsaturated or monounsaturated fat on plasma lipids in free-living young adults. *Eur. J. Clin. Nutr*. **55**: 908–915.
- Shepherd, J., C. J. Packard, S. M. Grundy, D. Yeshurun, A. M. Gotto, Jr., and O. D. Taunton. 1980. Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man. *J. Lipid Res*. **21**: 91–99.
- Sola, R., C. Motta, M. Maille, M. T. Bargallo, C. Boinsier, J. L. Richard, and B. Jacotot. 1993. Dietary monounsaturated fatty acids enhance cholesterol efflux from human fibroblasts. Relation to fluidity, phospholipid fatty acid composition, overall composition, and size of HDL3. *Arterioscler. Thromb*. **13**: 958–966.
- Sola, R., A. E. La Ville, J. L. Richard, C. Motta, M. T. Bargallo, J. Girona, L. Masana, and B. Jacotot. 1997. Oleic acid rich diet protects against the oxidative modification of high density lipoprotein. *Free Radic. Biol. Med*. **22**: 1037–1045.
- Brites, F., J. Verona, C. De Geitere, J. C. Fruchart, G. Castro, and R. Wikinski. 2004. Enhanced cholesterol efflux promotion in well-trained soccer players. *Metabolism*. **53**: 1262–1267.
- van der Gaag, M. S., A. van Tol, S. H. Vermunt, L. M. Scheek, G. Schaafsma, and H. F. Hendriks. 2001. Alcohol consumption stimulates early steps in reverse cholesterol transport. *J. Lipid Res*. **42**: 2077–2083.
- Rao, M. N., Q. H. Liu, P. Marmillot, L. B. Seeff, D. B. Strader, and M. R. Lakshman. 2000. High-density lipoproteins from human alcoholics exhibit impaired reverse cholesterol transport function. *Metabolism*. **49**: 1406–1410.
- Palmer, A. M., N. Murphy, and A. Graham. 2004. Triglyceride-rich lipoproteins inhibit cholesterol efflux to apolipoprotein (apo) A1 from human macrophage foam cells. *Atherosclerosis*. **173**: 27–38.
- Mweva, S., J. L. Paul, and M. Cambillau. 2006. Comparison of different cellular models measuring in vitro the whole human serum cholesterol efflux capacity. *Eur. J. Clin. Invest*. **36**: 552–559.
- Berrougui, H., M. Cloutier, M. Isabelle, and A. Khalil. 2006. Phenolic-extract from argan oil (*Argania spinosa* L.) inhibits human low-density lipoprotein (LDL) oxidation and enhances cholesterol efflux from human THP-1 macrophages. *Atherosclerosis*. **184**: 389–396.
- Uehara, Y., S. Miura, A. von Eckardstein, S. Abe, A. Fujii, Y. Matsuo, S. Rust, S. Lorkowski, G. Assmann, T. Yamada, et al. 2007. Unsaturated fatty acids suppress the expression of the ATP-binding cassette transporter G1 (ABCG1) and ABCA1 genes via an LXR/RXR responsive element. *Atherosclerosis*. **191**: 11–21.
- Sporstøl, M., S. A. Mousavi, W. Eskild, N. Roos, and T. Berg. 2007. ABCA1, ABCG1 and SR-BI: hormonal regulation in primary rat hepatocytes and human cell lines. *BMC Mol. Biol*. **8**: 5.