

Life Style Change and Reverse Cholesterol Transport in Obese Women

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Summary

HDL cholesterol resp. apolipoprotein A1 concentrations are tools to estimate individual CVD risk, although only a part of HDL particles participate in reverse cholesterol transport (RCT). This discrepancy was analyzed in life style change based on increase of physical activity and dietary counseling. Efflux of cholesterol from pre-labeled macrophages to plasma acceptors of tested individuals was used as an RCT measure. Changes of lipoprotein parameters, glucose, fasting insulin concentrations and RCT were analyzed in 15 obese women after 9-week intervention consisted of 5 sessions of increased physical activity per week. Controlled increase in physical activity for 9 weeks induced a decrease of body weight averaging 9 kg (ranged from 2.3 to 15.5 kg). The intervention leads to significant decreases of triglycerides, apoprotein A1 and apoprotein B concentration, whereas total cholesterol, LDL cholesterol and HDL cholesterol did not change significantly. The increase of RCT was not significant, but there was highly significant negative correlation between individual decrease of body weight and an increase of RCT. Significant increase of RCT was found in 13 persons with a weight reduction more than 3.5 kg. Substantial weight loss is necessary to increase RCT.

Key words

Reverse cholesterol transport • Cholesterol efflux • Lipoprotein metabolism • Life style modification

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Introduction

An increased risk of cardiovascular disease (CVD) in overweight and obese individuals is partly mediated by a low concentration of high density lipoproteins as the key biochemical feature of atherosclerosis is the accumulation of excess cholesterol in arterial intimal macrophages (Ross 1986). Although HDL particles display a spectrum of anti-atherosclerotic properties (anti-inflammatory, LDL oxidation protection, anti-apoptotic, antithrombotic, etc. (Von Eckardstein *et al.* 2001, Murphy *et al.* 2008)) their role in reverse cholesterol transport (RCT) from extrahepatic cells is probably the most important (Sviridov and Nestel 2002, Catalano *et al.* 2008).

Transfer of cholesterol excess from the cell is mediated by ABCA1, ABCG1 and SR-B1 cellular receptors. The monogenic defect (Tangier disease, Rust *et al.* 1999) as well as polygenic defects (Soro-Paavonen *et al.* 2007) of these receptors are able to influence RCT. On the other hand, RCT is also influenced by the capacity of intravascular acceptors – namely lipid poor HDL and large HDL particles – in different individuals and different statuses (Boadu *et al.* 2008, Marcel *et al.* 2008). It is very probable that for the majority of extrahepatic cells RCT is in equilibrium with LDL mediated transport to these cells and an increase of intravascular LDL concentration is accompanied by RCT acceleration (De Vries *et al.* 2005). A disequilibrium between the inflow of LDL cholesterol and its outflow from the cell leads to intracellular cholesterol cummulation. In monocytes located in the subendothelial space of arteries this cummulation is the main reason of their transformation into macrophages, residential macrophages and finally foam cells. This

process plays a pivotal role not only in atherogenesis but also in stability of already formed cells. The reduced expression of specific receptors (Mauldin *et al.* 2008) is responsible for reduced RCT as well as the possible change of acceptors that might influence CDC risk in obese individuals (Marcel *et al.* 2008). In obese individuals, a high concentration of triglyceride rich lipoproteins which is also a common phenotype in these individuals might participate in regulation of RCT as it has been proven that triglyceride rich particles directly inhibit cholesterol efflux from human macrophage foam cells (Palmer *et al.* 2004).

Although data in the literature is partly conflicting, there is a majority opinion that adequately increased physical activity is able to increase HDL cholesterol and also probably apolipoprotein A1 concentrations (Kelley *et al.* 2004). We have documented that an intervention of life style of obese individuals is able to decrease the volume of abdominal as well as subcutaneous fat followed by decrease of proinflammation status and increased HDL cholesterol concentration (Dvořáková-Lorenzová *et al.* 2006). In this study, a similar life style change producing weight reduction by a combination of physical activity and dietary counseling was applied to analyze the possible role of weight reduction to the direct measurement of RCT as HDL concentration need not be simply related to cholesterol efflux (Soro-Paavonen *et al.* 2007).

Methods

Subjects

Fifteen healthy obese female volunteers were recruited through advertisements in life style magazines and healthy life style web sites. Inclusion criteria were: self motivation, under 40 years of age, BMI>29 and no apparent illness or medication. The design of the study was approved by Ethical Committee of the Institute of Clinical and Experimental Medicine and informed consent was obtained from all study subjects before the beginning of the study.

Life style intervention

A 9-week intervention consisted of 5 sessions of increase physical activity per week, at minimum 3 sessions were conducted under controlled conditions at a fitness centre and other two sessions (non controlled) of brisk walking or bicycling were performed usually at the weekend. Every session lasted 60 minutes. All

participants obtained frequent dietary counseling based on their own 3-day dietary record. Substantial differences from the healthy diet (AHA) were identified in majority of subjects and it was successfully and positively influenced. Dietary counseling together with body weight record were repeated every second week throughout of the study.

Clinical biochemistry parameters

Blood specimens were obtained after 12 hrs overnight fasting into a vacutainer tube at the beginning and at the end of the study after 9 weeks of intervention. After aggregation at room temperature, the serum was separated at 3500 RPM (10 minutes), portioned, frozen and stored at -80 °C. Aliquots of both serum were analyzed on the same day after whole experiment was completed. Concentration of triglyceride and total and LDL cholesterol were determined by Roche Diagnostica kits. HDL cholesterol was analyzed with the same kit after separation of apolipoprotein B containing particles by phosphotungstate method. Non-esterified fatty acids (NEFA) were measured by enzymatic test (Wako Chemicals GmbH., Neuss, Germany) and apoprotein A1 and apoprotein B by immunothurbidimetric method (Orion Diagnostica, Espoo, Finland). A Hitachi 920 autoanalyzer was used for all biochemical measurements and lipoprotein parameters and laboratory was under external quality control of CDC Atlanta, USA.

Physical data

Blood pressure was measured in a sitting position after 10 minutes rest and average of 3 measurements were used. Body weight was measured by calibrated balance with accuracy ± 0.1 kg. Waist circumference was measured at the umbilicus area.

Cholesterol efflux measurement

RCT was measured in triplicate using in vitro macrophages pre-labeled with ^{14}C cholesterol (Perkin Elmer Life Sciences). THP1 human monocytes were seeded into 24 well plates at a density 2×10^5 cells/ml and differentiated into macrophages by phorbol 12-myristate 13-acetate (100 ng/ml, Sigma Aldrich). Cholesterol efflux was analyzed after 240 min incubation in RPM1 medium containing 5 % of serum of studied subjects as acceptor. Cholesterol efflux was measured between 15 and 240 min. At the end of incubation, the media were centrifuged at 450 g at 4 °C to remove any floating cells and supernatant measured with scintillation liquid (described

in details Poledne *et al.* 2009).

Medium free cells were twice washed with ice-cold PBS with 0.1 % fatty acids free albumin. Cells were frozen for 1 hr and then lyzed with isopropanol-hexan. Aliquot of extract was centrifuged at 440 g, evaporated in scintillation vials and dissolved in scintillation liquid. Cholesterol efflux in % was calculated as radioactivity in the efflux medium divided by total radioactivity of cells and media and multiplied by 100. Absolute differences in triplicate were ± 1.20 % (S.D. 0.52), coefficient of variation of efflux assay was 9.8 %. The measurement of efflux was not corrected to efflux to albumin alone and then it might include also label carried by endogenously synthesized apolipoprotein E. This part of efflux should not vary within wells and individuals.

Statistical method

All results are expressed as mean \pm standard deviation. The difference between baseline data and data at the end of intervention were evaluated using a pair t-test. Correlation of parameters was analyzed by simple linear method.

Results

The life style intervention of 15 young obese women produced a substantial drop of body weight of more than 7 kg on average (Table 1). This decrease represents the change of body weight for almost 8 % and it is very similar to our previous large study (Dvořáková-Lorenzová *et al.* 2006). Unfortunately, in this present experiment we were able to detect much higher variability inside of this intervened group and maximal body weight decrease was 15.5 kg whereas on the other side one volunteer decreased her body weight only for 2.3 kg (after 9 weeks intervention consisting of increased physical activity and dietary change). It was not apparent from individual records of volunteers either for exercise sessions or of energy intake that differences in body weight reduction are related to any of these measures.

Body waist and hips circumference decreased highly significantly with a rather modest change of waist/hip ratio. These changes in anthropometric parameters were not followed by any significant decrease of non-esterified fatty acids whereas this parameter was significantly influenced by similar type of intervention in our earlier experiments (Dvořáková-Lorenzová *et al.* 2006).

Although insulin sensitivity analyzed as a

Table 1. Anthropometric parameters at the baseline and after intervention.

	Baseline	After life style intervention
Weight (kg)	91.73 \pm 9.57	84.59 \pm 9.62*
BMI (kg.m ⁻²)	33.18 \pm 2.03)	30.57 \pm 2.07*
Waist (cm)	101.94 \pm 7.7)	95.31 \pm 7.18*
Hip (cm)	118 \pm 5.96)	112.53 \pm 5.13*

Data are mean \pm S.D. * significantly different $p < 0.0001$.

change of concentration of fasting insulin increased substantially at the end of experiment (Table 2), the decrease of fasting glucose concentration was only at the border line of statistical significance ($p = 0.056$).

There were no significant changes either in the concentration of total cholesterol, LDL cholesterol or HDL cholesterol concentrations when baseline data were compared to data obtained at the end of intervention. On the contrary, there was a slight but significant decrease of both apoprotein B and apoprotein A1 concentration. These changes were related to change of total fat consumption calculated from dietary records (data not presented).

Cholesterol efflux increased only for less than 1 percentage point (i.e. 6.3 % of baseline value) and this difference was not statistically significant (Table 2). A surprisingly very close correlation was found between the decrease of body weight and the individual change of cholesterol efflux after intervention. Volunteers with the smallest weight reduction also displayed the smallest change of cholesterol efflux after intervention (Fig. 1). Based on this information, an additional calculation was completed in which 2 volunteers with the lowest body weight change were excluded. When these two volunteers were omitted, cholesterol efflux increased for 1.8 percentage points (i.e. 14 % of baseline value) and the calculated statistical significance of cholesterol efflux became significant (Table 2).

Discussion

Low physical activity and obesity have been shown to increase CHD risk and numerous studies have analyzed the effects of increased exercise and body weight reduction on the different levels of risk factors of premature atherosclerosis and CHD. The positive effects

Table 2. Biochemical data at the baseline and after intervention.

Total cholesterol (mmol/l)	4.55 ± 0.82	4.26 ± 0.67	n.s.
LDL cholesterol (mmol/l)	2.90 ± 0.78	2.71 ± 0.69	n.s.
HDL cholesterol (mmol/l)	1.36 ± 0.41	1.33 ± 0.39	n.s.
Triglycerides (mmol/l)	1.59 ± 0.76	1.33 ± 0.57	p<0.05
Apoprotein B (gr/l)	0.91 ± 0.20	0.83 ± 0.19	p<0.05
Apoprotein A1 (gr/l)	1.41 ± 0.35	1.33 ± 0.31	p<0.05
NEFA (mmol/l)	0.79 ± 0.39	0.86 ± 0.20	n.s.
Glucose (mmol/l)	5.50 ± 0.56	5.19 ± 0.31	p=0.055
Fasting insulin (U/l)	10.12 ± 3.40	6.02 ± 2.12	p<0.001
Lipoprotein lipase (mmolFFA/h/lPHP)	2.31 ± 0.81	2.99 ± 1.06	p<0.02
Cholesterol efflux I (%) n=15	12.87 ± 2.39	13.68 ± 1.89	n.s.
Cholesterol efflux II (%) n=13	12.65 ± 1.62	14.42 ± 1.42	p<0.05

Data are mean ± S.D.

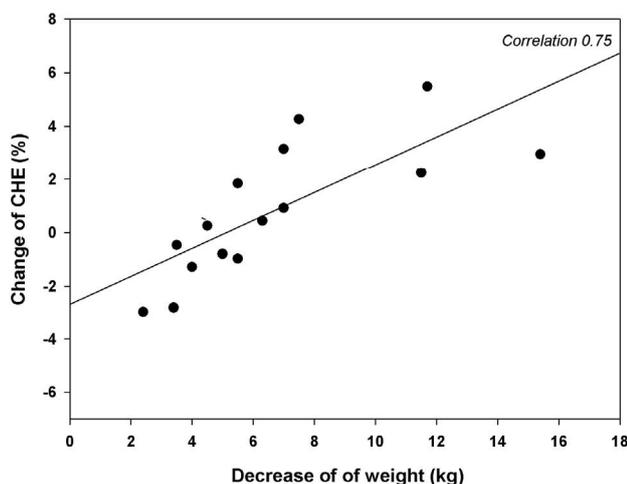


Fig 1. Relationship between the change of weight and change of cholesterol efflux (CHE) after nine weeks of intervention included increase in physical activity and switch to rational diet.

of this type of intervention are partly mediated by a change in lipoprotein profile. Different studies analyzing direct effect of exercise on HDL cholesterol or apoA1 concentrations have not consistently shown positive effects (Kelley *et al.* 2004). There is not general agreement on the effect of exercise on HDL concentration and composition of HDL particle size and their potential to clear an excess of cholesterol from extrahepatic cells. It has been suggested that long term endurance physical exercise is required to reach a significant increase of HDL cholesterol and apoA1 concentration (Williams *et al.* 1990).

Although a substantial and highly significant decrease in body weight as well as in other anthropometric parameters has been documented in this study there was no significant change of HDL cholesterol

concentration. We suppose that the level of physical activity in our study was not sufficient to influence HDL concentration in spite of substantial decrease of body weight. Additionally, the total cholesterol and LDL cholesterol concentration were not changed. It must be considered that baseline data of our obese volunteers were in the normal range and are lower compared to a representative sample of the Czech population at the same age (data not shown) (Cífková *et al.* 2009).

Decrease of body weight by physical activity decreased significantly the fasting triglyceride concentration although it was also at the normal range at baseline. Our previous study shown that change of triglycerides after similar intervention differ in carriers of APOA5 gene (Suchánek *et al.* 2008) variants, nevertheless we did not determined genetic factors in this study. The significant decrease of triglycerides is in agreement with an increase of lipoprotein lipase activity, moderate decrease of fasting glucose concentration and a significant decrease of fasting insulin concentration. The effect of different level of physical activity on RCT has been documented in one study (Hoang *et al.* 2008), but on the contrary from the study presented, only the questionnaire data were used to compare to groups with different level of physical activity.

When the decrease of body weight and cholesterol efflux was correlated in all 15 volunteers, a highly significant correlation was found (Fig. 1). This might explain why an increase in RCT did not reached statistical significance. When data of two women with lowest change of body weight (2.1 and 3.2 kg respectively) were excluded, the difference between baseline and final mean of cholesterol efflux increased

and became statistically significant (Table 2).

We should address comment on great differences of weight reduction between our subjects (from 2.3 to 15.5 kg). There are three apparent possibilities for these differences: firstly different level of activities during two non-controlled session and during days without designed physical activities. Secondly we can suppose inter-individual metabolic differences, possibly in part genetic that may influence effect of our intervention. Thirdly we must admit that the diet was not fully controlled and subjects could differ in their compliance to dietary counseling.

It is possible to conclude that change of the life style of obese young individuals leads to decrease of

body weight and waist and hip circumference. There was also a trend to increase the efflux of cholesterol from macrophages as a measure of RCT. Nevertheless, it is necessary to decrease body weight for more than 4-5 % to reach a biologically significant increase in RCT.

Conflict of Interest

There is no conflict of interest.

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