Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 308 (2003) 29–34
BBRC
www.elsevier.com/locate/ybbrc

Calorie restriction in mice does not affect LDL reverse cholesterol transport in vivo

O. Stein,[a] Y. Dabach,[b] G. Halperin,[c] M. Ben-Naim,[a] and Y. Stein[b,*]

[a] *Department of Experimental Medicine and Cancer Research, Hebrew University-Hadassah Medical School, Israel*
[b] *Lipid Research Laboratory, Division of Medicine, Hadassah University Hospital, P.O.B. 12220, 91 120 Jerusalem, Israel*
[c] *Lipid Research Institute, Sheba Hospital, Tel-Hashomer, Israel*

Received 16 June 2003

Abstract

Calorie restriction (CR) prolongs life in animals, but may reduce plasma HDL, important in reverse cholesterol transport (RCT). The effect of CR, 60% of an ad libitum (AL) diet, on cholesterol removal from rectus femoris muscle injected with cationized LDL, was studied in C57BL male mice. RCT in vivo, on CR and AL diet, and cholesterol efflux from macrophages exposed to CR or AL sera, was similar, despite a 22% reduction in plasma HDL-cholesterol (HDL-C). In CR fed mice total cholesterol (TC) and phospholipid (T-PL) decreased by 32% and 38%, while HDL-C and HDL-PL decreased by 22% and 16% only, resulting in increased HDL-PL/T-PL ratio, which enhanced RCT. Partial re-feeding (CR-RF, 70% of AL) induced normalization of plasma lipids (excluding triglycerides), while HDL-PL/T-PL remained elevated. Thus, as CR did not interfere with RCT in vivo, it could possibly be beneficial to patients at risk for coronary heart disease.

Keywords: RCT; HDL; Triglyceride; Cholesterol efflux; Calorie restriction; Re-feeding; Macrophages

The observation that calorie restriction (CR) is associated with a marked increase in rodent longevity has been well documented [1–4] and studies have been directed towards the elucidation of the underlying mechanisms, such as oxidative stress [5,6]. Recently, CR was shown to retard significantly the development of atherosclerosis in apoE$^{-/-}$ mice [7]. In that study, this salutary effect was attributed to reduction of oxidative stress. However, there is mounting evidence that reverse cholesterol transport (RCT), in which high-density lipoproteins (HDL) play the pivotal role, is important in reduction of atherosclerosis in transgenic mice [8–11]. CR in humans [12,13] and monkeys [14–16] was shown to reduce significantly major risk factors for CHD, such as plasma triglycerides, LDL cholesterol, and obesity. However, in some human studies, CR was accompanied also by a decrease in HDL [12,13]. The major question is whether this reduction in HDL may interfere with RCT from atherosclerotic lesions and permit progression; more specifically, should such a therapeutic approach be recommended to subjects who may already have atherosclerotic involvement which is still not manifested clinically.

During the past few years, we have developed a model system in mice which permits quantitative evaluation of cholesterol loss from a depot created by injection of cationized LDL into the major thigh muscle (rectus femoris) under different experimental conditions [17–21]. In the present study, we applied this methodology to investigate the effect of CR on the rate of cholesterol clearance from the depot, which represents RCT in vivo.

Materials and methods

Animals and diet. C57BL/6 male mice from Jackson Labs were kept in the specific pathogen-free facility of the Hebrew University-Hadassah Medical School and fed a standard chow diet. At about 3 months of age, the mice were placed in individual cages and fed the ad

* Corresponding author. Fax: +972-2-643-4701.
E-mail address: ystein@hadassah.org.il (Y. Stein).

0006-291X/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/S0006-291X(03)01313-5

libitum diet (AL). The diet consisted of powdered standard chow formed into balls by the addition of gelatin dissolved in water [22]. The average food consumption of the entire cohort was estimated daily, by weighing the amount of food given and that remaining, during a period of two weeks. The amount of food consumed was taken as 100% and the calorie restriction (CR) diet was 60% of the AL diet (protocol I). In order to evaluate the effect of a less severe calorie restriction, mice were kept on the CR (60% of AL) for 4 weeks. Thereafter partial re-feeding was started (RF) and the CR was changed to 70% of AL diet (CR-RF) for an additional 4 weeks (protocol II). All mice were weighed at weekly intervals.

Lipoproteins. LDL were isolated from human plasma [23] and cationization was carried out according to Basu et al. [24]. Cationized LDL were labeled with [^{3}H]free cholesterol ([^{3}H]FC) [17]. Preparations for injection were concentrated to 20 mg cholesterol/ml and sterile filtered.

Studies in vivo. Injection of cat-LDL was as previously described [17]. Briefly, the mouse was anesthetized with ketamine and acetopromazine, and 10 μl cat-LDL (200 μg cholesterol) was injected, through a small incision in the skin, into the rectus femoris muscle of the right leg, using a Hamilton syringe with a 1/2 in.-24 gauge needle. The needle was kept in place for 1 min and withdrawn slowly; any liquid that appeared was blotted and counted and the incision sutured. Eight and 12 days after injection, which was given on day 37 or 41 of the experiment, the mice were anesthetized, blood was drawn from the aorta, and the rectus femoris muscle (ca. 150 mg), on the injected and the contralateral side, was removed for estimation of endogenous cholesterol content. The muscle was minced and homogenized with 2×1 ml methanol, followed by 2×1 ml chloroform. Liver, spleen, and kidneys were also removed and weighed.

Analytical procedures. For determination of cholesterol in muscle, stigmasterol was added as an internal standard and the homogenate was left overnight at room temperature to allow lipid extraction. The lipid extract was brought to chloroform–methanol (2:1) and purified [25]. Chloroform extracts of muscle were taken for determination of radioactivity and of cholesterol mass by high-performance liquid chromatography[17].

Endogenous and exogenous cholesterol in muscle. Total cholesterol determined in the non-injected contralateral muscle in each animal was expressed per 100 mg wet weight. The mean of all determinations was designated "endogenous cholesterol." The value for "exogenous cholesterol" in the injected muscle was obtained after subtraction of the endogenous cholesterol [17].

Serum lipids. Total and high-density lipoprotein (HDL)-cholesterol were determined by an enzymatic procedure using a Boehringer kit. HDL-cholesterol was measured on the supernatant after dextran sulphate precipitation of serum, using a Beckman ultracentrifuge rotor TLA 100. Serum and HDL-phospholipids were determined using a Sentinel CH kit (Milano, Italy).

Cell culture. Peritoneal macrophages were obtained from C57BL/6 mice after intraperitoneal injection of thioglycollate [26]. The 10^6 cells/well were seeded in minimal essential medium (MEM) containing 10% fetal bovine serum. To label the cells with [^{3}H]cholesterol, the latter was added to the serum-containing medium (1 μCi/ml) [17]. To study [^{3}H]cholesterol efflux from the labeled macrophages, the medium was removed, the cells were washed with phosphate-buffered saline (PBS), and incubated with medium containing 0.2% bovine serum albumin, and then with serum-free medium at 37 °C for 15 min each. Thereafter the cells were incubated for 5 or 24 h with serum-free medium containing 2% or 5% serum derived from mice fed AL or CR diets. At the end of incubation, the medium was collected and centrifuged at 3000 RPM to remove nonadherent cells and radioactivity was determined on aliquots of medium and cells.

Materials. The culture medium and fetal bovine serum were from Gibco (New York, NY). [7α(*n*)-^{3}H]Cholesterol was from Amersham, UK. All reagents were of analytical grade from Sigma, St. Louis, MO.

Statistical evaluation. The results were presented as means ± SE. The difference between groups was tested with Student's *t* test.

Results

Body weight

The results obtained in the present study were from male C57BL/6 mice. A linear reduction in body weight occurred during the first 5 weeks of calorie restriction (60% of AL diet, protocol I); later on, the body weight stabilized (Fig. 1). The control mice fed the same diet but ad libitum showed a moderate increase in body weight of 8%. At the end of the experiment, after 7 weeks on the CR diet, the difference in weight between the two groups was 26% (Fig. 1). The mice were injected with cationized LDL labeled with [^{3}H]free cholesterol ([^{3}H]FC cat-LDL) and the experiment was terminated 8 or 12 days after injection, during which period the animals continued to receive CR or AL diet. The feeding of the CR diet resulted also in reduction in organ weights: 30% in liver, 45% in spleen, and 22% in kidney.

In the experiments with protocol II, partial re-feeding was instituted after initial 4 weeks of CR, the severity of CR was reduced from 60% of AL to 70%. During the first 4 weeks on CR, the mice lost 13.5% of body weight and regained most of their initial weight after 2 weeks on the 70% CR-RF regimen. In the following 2 weeks, their weight increased by 4.6%, reaching that of the AL mice (Fig. 2). Twelve days prior to termination of the experiment all mice were injected with [^{3}H]FC cat-LDL and the rate of clearance of the injected material was determined.

Serum lipids

In the CR mice there was a significant reduction in all serum lipids. Triglycerides were lower by 69%, total cholesterol by 32%, HDL-cholesterol (HDL-C) by 22%, total phospholipids (PL) by 38%, and HDL-phospholipids (HDL-PL) by 23% (Table 1). In the CR fed mice, HDL-C accounted for 70% of total cholesterol and HDL-PL for 88% of total serum PL. In the AL fed mice

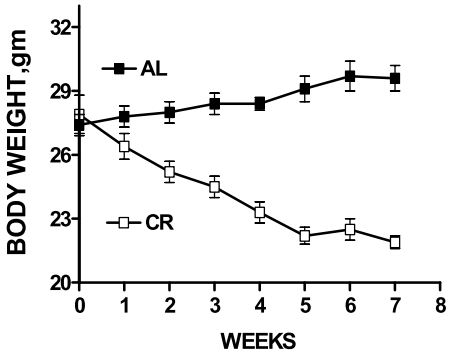

Fig. 1. Effect of calorie restriction (CR) diet on body weight, given for 49 days. The CR diet was 60% of the ad libitum (AL) diet fed to controls. Values are means ± SE of 12 male mice in each group.

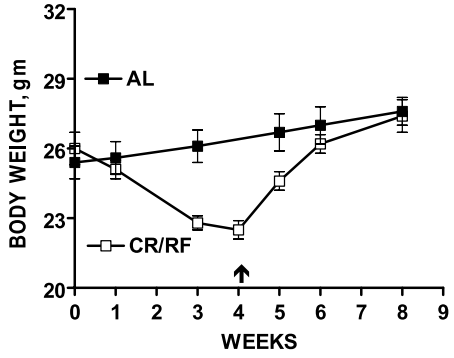

Fig. 2. Effect of calorie restriction (CR) and partial re-feeding (CR-RF) on body weight. The CR diet for the first 4 weeks was 60% of AL, while the CR-RF diet, given for the last 4 weeks, was 70% of AL. Black arrow shows start of CR-RF. Values are means ± SE of 10 male mice in each group.

the values were 60% and 65%, respectively. With protocol II, in which the mice were given 70% of AL during the last 4 weeks of the experiment, all plasma lipid concentrations, except for TG, increased and were not significantly different from the AL values (Table 1).

Clearance of [^{3}H]cholesterol and exogenous cholesterol mass from muscle

The clearance of the injected cat-LDL-C from the muscle 8 and 12 days after injection is summarized in Table 2. As reported previously [17], the clearance of the labeled free cholesterol ([^{3}H]FC) was more rapid than of total cholesterol mass. The cat-LDL contains 70% of cholesteryl ester (CE), which has to undergo hydrolysis prior to its release from the cell. Hydrolysis of CE is achieved by macrophages recruited to the muscle in response to the positive charges of cat-LDL [17,21]. In the present study, we compared the clearance of the injected lipoprotein cholesterol [^{3}H]FC or ECM from the muscle of the CR and the AL fed mice. Cholesterol removal was found to be similar in the CR and AL fed mice, at both time intervals examined (Table 2). The clearance of cat-LDL cholesterol was determined also in mice that were fed CR, 60% of AL, during the first 4 weeks, and then partially refed CR-RF, 70% of AL, for the next 4 weeks. Under these conditions, clearance of the injected cholesterol tended to be somewhat more rapid in the CR-RF mice, but the difference did not reach statistical significance (Table 2).

Table 2
Loss of exogenous cholesterol mass (ECM) from muscle after injection of [^{3}H]FC (free cholesterol) cat-LDL

Day after injection of cat-LDL	Diet	Protocol	Cholesterol in muscle (% of injected dose)	
			[^{3}H]Cholesterol	ECM
8	AL	I	32 ± 1.2	83 ± 8.1
8	CR	I	34 ± 3.2	83 ± 9.0
12	AL	I	14 ± 1.2	40 ± 2.1
12	CR	I	15 ± 1.7	42 ± 4.5
12	AL	II	19 ± 1.7	40 ± 1.6
12	CR-RF	II	16 ± 0.9	36 ± 1.8

AL, ad libitum; CR, calorie restriction 60% of AL; and CR-RF, calorie restriction 60% of AL, followed by partial refeeding at 70% of AL.

Values are means ± SE of 10–12 mice from either dietary group, per each time interval after injection of [^{3}H]FC cat LDL (200 μg cholesterol).

The differences between AL and CR or CR-RF fed mice were not statistically significant.

[^{3}H]Cholesterol efflux in cell culture

We also examined the capacity of serum from the mice, fed the CR or AL diet, for cholesterol efflux from peritoneal macrophages in cell culture. There was an increase in [^{3}H]cholesterol efflux from cells incubated with 2% or 5% of serum in culture medium and with time of incubation. However, the rate and extent of cholesterol removal was the same in the presence of CR or AL serum after 5 or 24 h of incubation at both serum concentrations (data not shown).

Discussion

Body weight and plasma lipids

Calorie restriction (CR) in the C57BL/6 mice resulted in 26% body weight reduction, similar to that reported

Table 1
Effect of calorie restriction on serum lipids

Diet	Protocol	TG (mg/dl)	TC (mg/dl)	HDL-C (mg/dl)	TPL (mg/dl)	HDL-PL (mg/dl)
AL	I	109 ± 7	106 ± 6	64 ± 6	269 ± 15	192 ± 9
CR	I	34 ± 3	72 ± 4	50 ± 5	166 ± 5	147 ± 12
AL	II	116 ± 7	102 ± 3	60 ± 4	280 ± 7	202 ± 9
CR-RF	II	50 ± 3	113 ± 3	63 ± 4	257 ± 12	213 ± 12

AL, ad libitum; CR, calorie restriction 60% of AL; and CR-RF, calorie restriction 60% of AL, followed by partial refeeding at 70% of AL.

TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; TP, total phospholipids; and HDL-PL, HDL-phospholipids.

Data are from mice presented in Figs. 1 and 2. Values are means ± SE of 10–12 mice on each dietary regimen. All plasma lipid values from AL mice of protocol I were statistically different from those of CR mice. $p < 0.01$, whereas the AL values of protocol II were not different from CR-RF values, except for TG $p < 0.01$.

in rats during a comparative period of feeding of a CR diet equivalent to 60% of AL value [27]. In apoE$^{-/-}$ mice fed 60% of an ad libitum diet, the body weight difference after a comparable period of time was similar to our results [7]. In the present study, the mice on the CR diet had significantly lower plasma triglycerides as compared to the controls, in analogy to the apoE$^{-/-}$ mice [7]. However, in the apoE$^{-/-}$ mice, which are hypercholesterolemic on a chow diet, there was no significant change in plasma total or HDL-cholesterol. These parameters decreased in the C57BL/6 mice on CR diet used presently, as was reported also for rats [28]. The mice on protocol II regained their body weight on CR-RF rapidly and reached that of the AL fed mice after 4 weeks only on 70% of the AL diet (CR-RF). The rapid weight gain in the partially refed mice could be due to adaptive changes in the efficiency of energy utilization, as shown previously in obese mice [29]. Moreover, exaggerated induction of lipogenic enzymes could have also contributed to the increase in adipose tissue in mice on CR-RF diet [30].

Plasma lipids were monitored in a long term study carried out in 2 species of nonhuman primates, cynomolgus monkeys fed 70% of the AL diet and rhesus monkeys fed 70% of a "western diet" for 4 years. In the rhesus monkeys there was a 50% reduction in plasma triglycerides, but no change in total or HDL-cholesterol; while in the cynomolgus monkeys, the CR did not change plasma lipids at all [15]. In another study in adult rhesus monkeys, calorie restriction resulted in a decrease in plasma triglycerides with no reduction in plasma cholesterol, but with a significant increase in HDL2b-cholesterol [14]. Calorie restriction was used also in humans, with the aim to induce weight loss in subjects that were markedly [31] or moderately obese [12]. Fifteen non-diabetic subjects with marked obesity (12 men and 3 women) were kept on 1000 kcal/day until they reached within 10% of the ideal body weight. During that time interval, plasma triglycerides decreased by 40%, plasma cholesterol by 15%, and HDL-C increased from 38 to 44 mg/dl [31]. Moderately obese healthy subjects, 7 men and 7 women, lost 9.6 and 10.4% of body weight, respectively, on 1000 kcal/day during 7 weeks of the study [12]. Plasma triglycerides in men decreased by 30% and in women by 39%; plasma cholesterol decreased by 19% in men and 11% in women, while HDL-cholesterol decreased by 12% and 9%, respectively [12]. The effect of CR in normal healthy non-obese subjects was studied in 4 men and 4 women who were sealed inside Biosphere 2 for a period of 2 years [13]. This unique study was initiated to evaluate the effect of dietary restriction on plasma lipids and lipoproteins in humans. The average energy deficit was about −1260 kJ/day in women and 2200 kJ/day in men; their normal body mass index decreased by 13% in women and 19% in men during the first 6 months. The average total plasma cholesterol of both sexes decreased by 36%, TG by 42%, LDL-C by 45%, and HDL-C by 57% [13]. The decrease in total plasma cholesterol may be due, in part, to suppression of endogenous cholesterol synthesis, as demonstrated in overweight men on CR diet [32]. In the present study, the restitution of body weight by partial re-feeding was accompanied also by an increase in all plasma lipids, except for triglyceride. HDL-PL accounted for 82% of total PL, and thus, the ratio of HDL-PL/TPL in the partially refed mice was still higher than in AL fed mice. Similar observations were made in humans after the termination of the Biosphere2 Study [13].

Calorie restriction and atherosclerosis

The subject of CR and atherosclerosis was studied most extensively in monkeys over a period of 4 years, but the study *failed to demonstrate* any difference in the extent of atherosclerotic lesions in the abdominal aorta between calorie restricted and control cynomolgus monkeys [15]. On the other hand, CR was shown to reduce atherosclerosis in apoE$^{-/-}$ mice [7]. Since CR resulted also in a significant decrease in plasma lipid peroxides, aortic superoxide and peroxide products, the authors suggested that reduction of oxidative stress may contribute to the anti-atherogenic effect of CR diet [7]. This hypothesis was tested in monkeys by comparing oxidizability of LDL in animals fed the calorie restricted diet and an AL diet. However, no consistent differences in the in vitro LDL oxidizability were observed in two monkey species on CR or control diets [33].

Calorie restriction and RCT

Another important atheroprotective mechanism is RCT, the first step of which is cholesterol efflux from cells [8]. In the present study, we compared cholesterol efflux in vivo in mice on CR or AL diet and there was no significant difference between the two groups. These results were surprising at first because calorie restriction did result in a 22% decrease in plasma HDL-C, which is important for cholesterol removal. However, a closer look at the plasma total and HDL-cholesterol levels reveals that the decrease in total cholesterol in mice on CR diet exceeded that in HDL-C, resulting in a higher HDL-C/TC ratio, i.e. 0.70, as compared to 0.60 in controls. This was even more apparent with respect to phospholipids in which HDL-PL/T-PL ratio was 0.89 on CR diet and 0.65 on control diet. Since the primary acceptor of cellular cholesterol is an apoAI phospholipid complex [34], HDL enriched in phospholipid may have a greater capacity for cholesterol efflux, and hence may compensate for the small decrease in plasma HDL-C on a CR diet.

It is relevant that a 30% reduction in plasma HDL in postmenopausal monkeys treated with Tibolone did not lower the cholesterol efflux potential of their serum, studied in cell culture, and even a 50% reduction of HDL decreased cholesterol efflux by 14% only [35]. In a study of postmenopausal women treated with Tibolone, plasma HDL-C was reduced by 14% and (Lp) A-I by 20%, but there was no reduction in the capacity of plasma to induce cholesterol efflux from either cultured human fibroblasts or FU5AH hepatoma cells [36]. Similar results were obtained also in the present study with sera of mice on CR and AL diets with respect to cholesterol efflux from macrophages.

In conclusion, this study shows for the first time that calorie restriction in mice did not decrease RCT in vivo, and did not affect cholesterol removal potential of serum from macrophages in culture. Thus, it appears that a small reduction in plasma HDL-C is not detrimental to reverse cholesterol transport if it is accompanied by a rise in HDL-C/TC and HDL-PL/T-PL ratio. If the results in mice can be extrapolated to humans, it would seem that the CR, because of its CHD risk reducing capacity and the non interference with cholesterol efflux from the periphery, could be of benefit for those patients.

Acknowledgment

Our thanks are extended to Mrs. Nina Kellman for her indefatigable help and patience in the preparation of the manuscript for publication.

References

[1] C.M. McKay, M.F. Crowell, L.A. Maynard, The effect of retarded growth upon the length of the lifespan and ultimate body size, J. Nutr. 10 (1935) 63–79.
[2] R. Weindruch, R.L. Walford, The Retardation of Aging and Disease by Dietary Restriction, Charles Thomas, Springfield, IL, 1988.
[3] B.P. Yu (Ed.), Modulation of Aging Processes by Dietary Restriction, CRC Press, Boca Raton, 1994.
[4] M.A. Lane, J. Mattison, D.K. Ingram, G. Roth, Caloric restriction and aging in primates: Relevance to humans and possible CR mimetics, Microsc. Res. Tech. 59 (2002) 335–338.
[5] R.S. Sohal, S. Agarwal, M. Candas, M.J. Forster, H. Lal, Effect of age and caloric restriction on DNA oxidative damage in different tissues of C57BL/6 mice, Mech. Ageing Dev. 76 (1994) 215–224.
[6] A. Lass, B.H. Sohal, R. Weindruch, M.J. Forster, R.S. Sohal, Caloric restriction prevents age-associated accrual of oxidative damage to mouse skeletal muscle mitochondria, Free Radic. Biol. Med. 25 (1998) 1089–1097.
[7] Z.M. Guo, F. Mitchell-Raymundo, H. Yang, Y. Ikeno, J. Nelson, V. Diaz, A. Richardson, R. Reddick, Dietary restriction reduces atherosclerosis and oxidative stress in the aorta of apolipoprotein E-deficient mice, Mech. Ageing Dev. 123 (2002) 1121–1131.
[8] O. Stein, Y. Stein, Atheroprotective mechanisms of HDL, Atherosclerosis 144 (1999) 285–301.
[9] A.D. Attie, J.P. Kastelein, M.R. Hayden, Pivotal role of ABCA1 in reverse cholesterol transport influencing HDL levels and susceptibility to atherosclerosis, J. Lipid Res. 42 (2001) 1717–1726.
[10] S.B. Joseph, E. McKilligin, L. Pei, M.A. Watson, A.R. Collins, B.A. Laffitte, M. Chen, G. Noh, J. Goodman, G.N. Hagger, J. Tran, T.K. Tippin, X. Wang, A.J. Lusis, W.A. Hsueh, R.E. Law, J.L. Collins, T.M. Willson, P. Tontonoz, Synthetic LXR ligand inhibits the development of atherosclerosis in mice, Proc. Natl. Acad. Sci. USA 99 (2002) 7604–7609.
[11] J. Repa, D.J. Mangelsdorf, The liver X receptor gene team: Potential new players in atherosclerosis, Nat. Med. 8 (2002) 1243–1248.
[12] J. Zimmerman, N.A. Kaufmann, M. Fainaru, S. Eisenberg, Y. Oschry, Y. Friedlander, Y. Stein, Effect of weight loss in moderate obesity on plasma lipoprotein and apolipoprotein levels and on high density lipoprotein composition, Arteriosclerosis 4 (1984) 115–123.
[13] R.B. Verdery, R.L. Walford, Changes in plasma lipids and lipoproteins in humans during a 2-year period of dietary restriction in Biosphere 2, Arch. Intern. Med. 158 (1998) 900–906.
[14] R.B. Verdery, D.K. Ingram, G.S. Roth, M.A. Lane, Caloric restriction increases HDL2 levels in rhesus monkeys (*Macaca mulatta*), Am. J. Physiol. 273 (1997) E714–E719.
[15] W.T. Cefalu, J.D. Wagner, A.D. Bell-Farrow, I.J. Edwards, J.G. Terry, R. Weindruch, J.W. Kemnitz, Influence of caloric restriction on the development of atherosclerosis in nonhuman primates: progress to date, Toxicol. Sci. 52 (1999) 49–55.
[16] M.A. Lane, E.M. Tilmont, H. De Angelis, A. Handy, D.K. Ingram, J.W. Kemnitz, G.S. Roth, Short-term calorie restriction improves disease-related markers in older male rhesus monkeys (*Macaca mulatta*), Mech. Ageing Dev. 112 (1999) 185–196.
[17] O. Stein, Y. Dabach, G. Hollander, M. Ben-Naim, G. Halperin, E. Okon, Y. Stein, Cholesterol efflux in vivo from a depot of cationized LDL injected into a thigh muscle of small rodents, Atherosclerosis 133 (1997) 15–22.
[18] O. Stein, Y. Dabach, G. Hollander, M. Ben-Naim, G. Halperin, J.L. Breslow, Y. Stein, Delayed loss of cholesterol from a localized lipoprotein depot in apolipoprotein A-I-deficient mice, Proc. Natl. Acad. Sci. USA 94 (1997) 9820–9824.
[19] O. Stein, G. Halperin, Y. Dabach, G. Hollander, M. Ben-Naim, Y. Stein, Effect of atherogenic diet on reverse cholesterol transport in vivo in atherosclerosis susceptible (C57BL/6) and resistant (C3H) mice, Atherosclerosis 156 (2001) 307–313.
[20] O. Stein, Y. Dabach, G. Hollander, M. Ben-Naim, G. Halperin, Y. Stein, Reverse cholesterol transport in mice expressing simian cholesteryl ester transfer protein, Atherosclerosis 164 (2002) 73–78.
[21] O. Stein, Y. Dabach, M. Ben-Naim, G. Halperin, I.F. Charo, Y. Stein, In CCR2$^{-/-}$ mice monocyte recruitment and egress of LDL cholesterol in vivo is impaired, Biochem. Biophys. Res. Commun. 300 (2003) 477–481.
[22] S.D. van Leeuwen, O.B. Bonne, Y. Avraham, E.M. Berry, Separation as a new animal model for self-induced weight loss, Physiol. Behav. 62 (1997) 77–81.
[23] R.J. Havel, H.A. Eder, J.H. Bragdon, The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum, J. Clin. Invest. 34 (1955) 1345–1353.
[24] S.K. Basu, J.L. Goldstein, R.G.W. Anderson, M.S. Brown, Degradation of cationized low density lipoprotein and regulation of cholesterol metabolism in homozygous familial hypercholesterolemia fibroblasts, Proc. Nat. Acad. Sci. USA 73 (1976) 3178–3182.
[25] J. Folch, M. Lees, G.H. Sloan-Stanley, A simple method for the isolation and purification of total lipids from animal tissue, J. Biol. Chem. 226 (1957) 497–509.
[26] G. Friedman, T. Chajek-Shaul, R. Gallily, O. Stein, E. Shiloni, J. Etienne, Y. Stein, Modulation of lipoprotein lipase activity in

mouse peritoneal macrophages by recombinant human tumor necrosis factor, Biochim. Biophys. Acta 963 (1988) 201–207.
[27] R.W. Hardy, K.A. Meckling-Gill, J. Williford, R.A. Desmond, H. Wei, Energy restriction reduces long-chain saturated fatty acids associated with plasma lipids in aging male rats, J. Nutr. 132 (2002) 3172–3177.
[28] Y.S. Choi, S. Goto, I. Ikeda, M. Sugano, Age-related changes in lipid metabolism in rats: the consequence of moderate food restriction, Biochim. Biophys. Acta 963 (1988) 237–242.
[29] A.G. Dulloo, R. Calokatisa, Adaptation to low calorie intake in obese mice: contribution of a metabolic component to diminished energy expenditures during and after weight loss, Int. J. Obes. 15 (1991) 7–16.
[30] N.M. Morton, M.C. Holmes, C. Fievet, B. Staels, A. Tailleux, J.J. Mullins, J.R. Seckl, Improved lipid and lipoprotein profile, hepatic insulin sensitivity, and glucose tolerance in 11beta-hydroxysteroid dehydrogenase type 1 null mice, J. Biol. Chem. 276 (2001) 41293–41300.
[31] R.N. Wolf, S.M. Grundy, Influence of weight reduction on plasma lipoproteins in obese patients, Arteriosclerosis 3 (1983) 160–169.
[32] M. Di Buono, J.S. Hannah, L.I. Katzel, P.J. Jones, Weight loss due to energy restriction suppresses cholesterol biosynthesis in overweight, mildly hypercholesterolemic men, J. Nutr. 129 (1999) 1545–1548.
[33] W.T. Cefalu, J.G. Terry, M.J. Thomas, T.M. Morgan, I.J. Edwards, L.L. Rudel, J.W. Kemnitz, R. Weindruch, In vitro oxidation of low-density lipoprotein in two species of nonhuman primates subjected to caloric restriction, J. Gerontol. A 55 (2000) B355–B361.
[34] C.J. Fielding, P.E. Fielding, Molecular physiology of reverse cholesterol transport, J. Lipid Res. 36 (1995) 211–228.
[35] T.S. Mikkola, M.S. Anthony, T.B. Clarkson, R.W. St Clair, Serum cholesterol efflux potential in postmenopausal monkeys treated with tibolone or conjugated estrogens, Metabolism 51 (2002) 523–530.
[36] A. von Eckardstein, D. Crook, J. Elbers, J. Ragoobir, B. Ezeh, F. Helmond, N. Miller, H. Dieplinger, H.C. Bennink, G. Assmann, Tibolone lowers high density lipoprotein cholesterol by increasing hepatic lipase activity but does not impair cholesterol efflux, Clin. Endocrinol. (Oxford) 58 (2003) 49–58.