

Changes on the Physiological Lactonase Activity of Serum Paraoxonase 1 by a Diet Intervention for Weight Loss in Healthy Overweight and Obese Women

Kazuhiko Kotani^{1,2,*}, Naoki Sakane¹, Yoshiko Sano¹, Kokoro Tsuzaki¹, Yukiyo Matsuoka¹, Kahori Egawa³, Makiko Yoshimura³, Chika Horikawa³, Yoshinori Kitagawa³, Yoshinobu Kiso³, Satoshi Kimura⁴, John Schulze⁵, Jennifer Taing⁵, and Alejandro Gugliucci⁵

¹Division of Preventive Medicine, Clinical Research Institute for Endocrine and Metabolic Disease, National Hospital Organization Kyoto Medical Center, Kyoto 612-8555, Japan

²Department of Clinical Laboratory Medicine, Jichi Medical University, Tochigi 329-0498, Japan

³Institute for Health Care Science, Suntory Ltd. Research Center, Osaka 618-0024, Japan

⁴Department of Laboratory Medicine and Central Clinical Laboratory, Showa University Northern Yokohama Hospital, Yokohama 224-8503, Japan

⁵Glycation, Oxidation and Disease Laboratory, Touro University-California, Vallejo, CA 94592, United States

Received 23 March, 2009; Accepted 15 June, 2009

Summary Low caloric diet (LCD) is used for weight loss. Paraoxonase 1 (PON-1) is associated with the antioxidant functions of high-density lipoprotein (HDL). Among limited data on the relationships between obesity and PON-1, there has been no study on the effects of a stand-alone LCD on the physiological lactonase activity of PON-1. We investigated the prospective effects of LCD intervention (2 months) for weight loss on serum PON-1 activities (lactonase, arylesterase [mono-esterase] and tri-esterase) and HDL cholesterol (HDL-C), and their association with low-density lipoprotein cholesterol (LDL-C) in overweight and non-morbidly obese but otherwise healthy women ($n = 30$; mean age, 50.3 years; mean body mass index [BMI], 28.5 kg/m²). In addition to the data such as BMI, blood pressure, blood glucose and lipids, PON-1 activities were examined between pre- and post-intervention. The intervention reduced all metabolic outcomes, and PON-1 lactonase activity (determined with 5-[thiobutyl]butyrolactone) significantly decreased by 6.1%, paralleled by arylesterase (by 7.3%) and tri-esterase (by 7.8%). In multiple regression analysis, the percent change of PON-1 lactonase was significantly, positively and independently correlated to that of LDL-C ($\beta = 0.51$), HDL-C ($\beta = 0.40$), and BMI ($\beta = 0.37$). Our results showed that the solo diet treatment on weight loss might reduce serum PON-1 lactonase activity with reduced HDL-C and LDL-C. The relationship between the lactonase and LDL-C may be adaptive, plausibly hypothesizing less need for PON-1 activity as an antioxidant property to protect lipoproteins. Further research is needed to confirm this prediction.

Key Words: low caloric diet, low-density lipoprotein, high-density lipoprotein, weight reduction, BMI

*To whom correspondence should be addressed.

Tel: +81-285-58-7386 Fax: +81-285-44-9947

E-mail: kazukotani@jichi.ac.jp

Introduction

Obesity is a crucial risk factor in the development of its co-morbid diseases such as metabolic syndrome and atherosclerotic disorder including cardiovascular disease (CVD), and the increased prevalence of obesity is a major problem in many countries [1]. Impaired high-density lipoprotein (HDL) antioxidant defenses in obese people, such as a decreased activity of the HDL-associated enzyme, paraoxonase 1 (PON-1), have recently received increased attention [2]. PON-1, an esterase carried by HDL, is known to exert a protective effect against oxidative damage of cells and lipoproteins, playing an anti-inflammatory and anti-atherogenic role [2–4]. In fact, a low circulating PON-1 activity has been reportedly associated with obesity, metabolic syndrome and CVD [2, 5–7].

Low caloric diet (LCD) is one of the treatment modalities adopted as a part of lifestyle management on weight loss, leading to the improvement of metabolic and cardiovascular conditions [8, 9]. A few studies have addressed the changes in circulating PON-1 activity in the treatment intervention for obesity [10–12]. These previous results are not always consistent. In one, weight loss by gastric banding significantly increased PON-1 activity in morbidly obese patients [10]. In another study with obese patients treated with orlistat (an anti-obesity drug), PON-1 activity was significantly increased [11]. Weight reduction via a combination of exercise and diet energy restriction significantly reduced PON-1 [12]. As mentioned above, HDL is widely recognized to be an anti-atherogenic lipoprotein. Whereas the influence of calorie restriction diet for weight loss on lipid profiles, circulating HDL cholesterol (HDL-C) in particular, is reportedly controversial, HDL-C has been known to be often reduced in short-term diets [13–17]. The clinical significance and detailed mechanism of this is presently unclear, so accumulating data are required. Turnover studies have documented the changes in clearance of HDL-C during dietary modification [18], and the reduction in chylomicron-derived HDL formation following caloric (lipid) restriction diet is discussed as an alternate explanation [14]. There is an opinion that the diet-induced HDL-C drop does not increase CVD risk [19]; however, whether this diet-induced reduction of HDL-C is beneficial/harmful remains debatable.

One of the physiological roles of HDL involves the action of PON-1 [2, 4], and to evaluate a functional aspect of HDL in measuring PON-1 activity may yield further insights on this issue. The presence of dysfunctional HDL may, at the same HDL-C levels, signify quite different outcomes, and the same may be said about inflammatory/anti-inflammatory HDL. PON-1 hydrolyzes lipid peroxides, imparting protection to low-density lipoprotein (LDL) against oxidation [4, 6]. Although conflicting results exist [20], a low PON-1 activity generally implies their reduced ability to protect

LDL from oxidation, resulting in increased oxidized LDL [6]. There is a positive association between oxidized LDL and LDL cholesterol (LDL-C) concentrations [20], and a LCD treatment may have the beneficial effects of reducing LDL-C levels. Therefore, when we assess the effects of LCD on PON-1, its association with the beneficial changes of LDL-C is an important factor to consider, that is one aim of the present study.

Moreover, the previous studies to observe the association between weight loss and PON-1 had two main limitations. First, none has addressed the effects of LCD as a stand-alone treatment on PON-1 activity. Interventions by a combination of exercise and diet, or diet and orlistat, could make it difficult to determine which aspect of the intervention is responsible for the change of PON-1 (or HDL-C) and to what extent. In particular, when a diet treatment is combined with exercise, HDL-C metabolism can be modified [21, 22] by the improvement of skeletal muscle/liver and insulin homeostasis through exercise [23, 24]. One of our study aims was thus to investigate the effects on PON-1 of an intervention by stand-alone ‘LCD’ to generate results that are easier to interpret and accordingly more meaningful in practice. The second limitation of the previous studies is that they have employed substrates that can not necessarily reflect the physiological activity of PON-1, which is recently regarded primarily as a lactonase [25, 26]. Hence, measuring the lactonase would provide news in research on the diet-PON-1 association.

With this background in mind, the present study investigated, in a population of overweight and not-morbidly obese but otherwise healthy subjects, the prospective effects of a solo LCD intervention for weight loss on the physiological lactonase activity of serum PON-1. We also addressed its association with changes in LDL-C, HDL-C and other clinical outcomes. Taking into account the gender difference in HDL-C metabolism as a powerful confounder [16, 27], our work was restricted only to a female cohort.

Subjects and Methods

In total, 30 healthy and free-living Japanese women, aged 33 to 63 (mean age, 50.3 ± 8.5) years, with an overweight/obesity level of body mass index (BMI) (mean, 28.5 ± 3.3 kg/m²; range, 24.3 to 38.6) participated in this study. Exclusion criteria were as follows: the use of medications and nutrient supplements, pregnancy, current smoking, alcohol abuse, psychological contraindications as determined by study investigators, or known hypersensitivity to any of the ingredients of the formula. The study was approved by the Ethics committee of Kyoto Medical Center, and all subjects gave informed consent.

Concerning the LCD prescription, during the study intervention period, subjects were placed on the LCD (5023 kJ/

day) (consisting of protein, 47%; lipid, 7%; carbohydrate, 40%) with meal replacement (Diet's™, Suntory Co. Ltd., Osaka, Japan) every dinner for 2 months. The subjects were also instructed by trained dietitians about the planning of nutritionally balanced diets, and the adherence was checked through dietary daily records. They were encouraged not to change usual physical activity during the intervention.

The subjects' data at baseline (pre-intervention) and the end of 2 months' study (post-intervention) were used. In an overnight fast, we confirmed the subjects' data on BMI, blood pressure (measured using a mercury sphygmomanometer), plasma glucose (enzymatic kinetic method, Shino-test Co. Ltd., Tokyo, Japan) and lipids such as total cholesterol (enzymatic kinetic method, Wako Pure Chemical Industries Co. Ltd., Tokyo, Japan) and triglyceride (enzymatic kinetic method, Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan). Both HDL-C and LDL-C were measured using a homogeneous assay (Daiichi Pure Chemicals Co. Ltd.).

Serum PON-1 lactonase activity was kinetically determined at 37°C using 5-(thiobutyl)butyrolactone (TBBL), a lactone substrate that is analogous to γ -nonanoic lactone, yet releases upon hydrolysis of the γ -butyrolactone ring, and recorded at 405 nm in a VERSA max (Tunable) Microplate Reader [25, 26]. Serum PON-1 tri-esterase activity was determined using paraoxon as a substrate, from the initial velocity of *p*-nitrophenol production at 37°C, and recorded at 405 nm [28]. Serum PON-1 arylesterase (mono-esterase) activity was determined using phenylacetate as a substrate and kinetically measured at 37°C by recording absorbance changes at 270 nm [28, 29].

Data are expressed as mean \pm standard deviation (SD). The level change between pre- and post-intervention in respective variables was tested by paired *t* test. To detect

the correlations between the percent change of PON-1, HDL-C and LDL-C, we used Pearson's rank coefficient test as well as multiple regression analysis controlling for the above variables and age. A $p < 0.05$ was considered significant.

Results

All subjects completed the present LCD intervention. The data on respective variables in pre- and post-intervention were listed in Table 1. During the intervention, most variables (except for systolic blood pressure which did not achieve a statistical significance level) were significantly reduced. Notably, PON-1 lactonase, as well as arylesterase and tri-esterase activities, were significantly reduced.

As listed in Table 2, in the simple correlation tests, the percent change of BMI or LDL-C was significantly and positively correlated to that of PON-1 lactonase activity, along with age. Multiple regression analysis on PON-1 lactonase activity, adjusted for age, BMI, LDL-C and HDL-C, revealed that the percent change of LDL-C, HDL-C, and BMI (the correlation degree ranked in this order) was significantly, positively, and independently correlated to that of lactonase activity. Furthermore, multiple regression analysis on the percent change of HDL-C, adjusted with age, BMI, LDL-C and PON-1, revealed that the percent change of BMI was significantly, inversely, and independently correlated, and PON-1 lactonase activity was significantly, positively, and independently correlated to HDL-C. Additionally, the results in the correlation tests on arylesterase or tri-esterase activity were similar with those in PON-1 lactonase activity (data not shown).

Table 1. Outcome variables before and after a low calorie diet intervention

Variables	Pre-intervention	Post-intervention	<i>p</i> value
Body mass index (kg/m ²)	28.5 \pm 3.3	26.2 \pm 3.3	<0.001**
Systolic blood pressure (mmHg)	128.7 \pm 15.6	125.0 \pm 15.7	0.062
Diastolic blood pressure (mmHg)	80.0 \pm 10.9	75.1 \pm 9.9	0.001**
Glucose (mmol/L)	5.1 \pm 0.6	4.8 \pm 0.5	<0.001**
Total cholesterol (mmol/L)	5.77 \pm 0.93	5.38 \pm 0.96	<0.001**
Triglyceride (mmol/L)	1.12 \pm 0.74	1.00 \pm 0.65	0.030*
HDL cholesterol (mmol/L)	1.64 \pm 0.37	1.56 \pm 0.31	0.049*
LDL cholesterol (mmol/L)	3.27 \pm 0.67	2.99 \pm 0.70	<0.001**
PON-1 tri-esterase (U/L) ^a	153.6 \pm 60.6	141.2 \pm 53.4	0.002**
PON-1 arylesterase (U/L) ^b	123.2 \pm 21.2	114.3 \pm 22.2	0.001**
PON-1 lactonase (U/L) ^c	147.2 \pm 20.2	138.2 \pm 17.3	<0.001**

Data are mean \pm SD. HDL: high-density lipoprotein, LDL: low-density lipoprotein, PON-1: paraoxonase 1. ^a PON-1 tri-esterase: activity measured with paraoxon, ^b PON-1 arylesterase (mono-esterase): activity measured with phenylacetate, ^c PON-1 lactonase: activity measured with 5-(thiobutyl)butyrolactone (TBBL). PON-1 tri-esterase activity decreased by 7.8%, arylesterase by 7.3% and lactonase activity by 6.1%, respectively. Significance level (paired *t* test between pre- and post-intervention): * $p < 0.05$, ** $p < 0.01$.

Table 2. Correlations between the percent changes of HDL cholesterol, LDL cholesterol, and PON-1 lactonase activity during the intervention period

Variables	<i>r</i> (<i>p</i> value)	β (<i>p</i> value)
<i>For</i> PON-1 lactonase activity		
Age (years)	0.463 (0.010*)	0.021 (0.906)
Body mass index (kg/m ²)	0.408 (0.025*)	0.366 (0.041*)
LDL cholesterol (mmol/L)	0.604 (<0.0001**)	0.509 (0.001**)
HDL cholesterol (mmol/L)	0.278 (0.137)	0.399 (0.017*)
<i>For</i> HDL cholesterol		
Age (years)	0.276 (0.140)	0.419 (0.028*)
Body mass index (kg/m ²)	-0.286 (0.125)	-0.606 (0.002**)
LDL cholesterol (mmol/L)	-0.044 (0.819)	-0.316 (0.103)
PON-1 lactonase activity (U/L)	0.278 (0.137)	0.522 (0.017*)

HDL: high-density lipoprotein, LDL: low-density lipoprotein, PON-1: paraoxonase 1. *r*: Pearson's rank correlation coefficient, β : multiple regression coefficient (adjusted for all listed variables). Significance level: **p*<0.05, ***p*<0.01.

Discussion

Our study displays the effects of short-term LCD intervention for weight loss on PON-1 physiological lactonase activity and HDL-C, and their association to LDL-C changes, in healthy overweight/obese women. So far, evidence on the influence of weight loss on PON-1 has been inconsistent [10–12], and this may be partly explained by the differences in intervention methods and populations. In our cohort, all measured variables including BMI, HDL-C and PON-1 were reduced, and the reduction of BMI showed a significant positive correlation to that of PON-1. A previous study has reported that a combination of exercise and diet could reduce PON-1 in subjects with the metabolic syndrome [12], though in this case, the element of exercise may make some differences in the HDL metabolism from a solo diet treatment [23, 24]. Another previous study has also implicated that a healthy diet, for instance focused on vegetables, might reduce PON-1, though this study was not necessarily for obese subjects [30]. Taken together, our results seem to raise the possibility that a diet treatment reduces PON-1. Whether the mechanism was due to metabolic changes of HDL-C [14, 18] or a reaction to diet- and weight loss-induced reduction of oxidation (as described later) remains to be determined presently, but at least, our study adds to the present knowledge in two main areas: 1) the results from a better defined cohort of a homogeneous population of healthy and overweight/obese (non-morbidly) individuals and a well-controlled intervention by diet alone, 2) the novelty of demonstrating a decrease in lactonase activity that parallels the mono-esterase and tri-esterase changes.

PON-1 catalyzes the hydrolysis of numerous substrates: lactones, thiolactones, esters and phospho-triesters, including paraoxon, from which its name is derived. Nevertheless,

only after many years of research, it has become apparent that PON-1 is actually a lactonase, catalyzing the hydrolysis and synthesis of several lactones [25, 26, 31]. The other activities, such as arylesterase and phospho-triesterase, might be simply promiscuous, although they are used in clinical laboratory research as a practical way of measuring PON-1 activities (seen in the previous articles on weight loss) and even the commercial kits have appeared on the market this year. The physiological substrates of PON-1 remain uncertain; however, they are presumably lactones in food components or derivatives of fatty acid oxidation chain reactions such as 5-hydroxyeicosatetraenoic acid lactone. These end-product lactones of fatty acid oxidation may be found in LDL, HDL or macrophages [25, 26].

Based on the current wisdom that PON-1 is a protective factor against CVD [5–7], the lowering of such PON-1 activities might be interpreted as a detrimental effect of the diet. However, we think that this has to be balanced by the beneficial effects produced by the diet on other well-known risk factors for CVD. In this regard, our study has found an association that may explain this apparent 'paradoxical' effect of the LCD intervention. Indeed, to the best of our knowledge, this is the first description that there is a significant relationship between the lowering of PON-1 activity (its lactonase or the others) and that of LDL-C. Namely, the correlation between PON-1 and LDL-C changes in the LCD intervention has not been previously reported, although whether this is a specific effect of LCD is unclear. Interestingly, from our study results, this relationship was not fully explained by the reduction in HDL-C. Shedding more light on the mechanism for the relationship between PON-1 and LDL-C is a future challenge, but we would suggest this hypothetical flow of events. PON-1 hydrolyzes lipid hydroperoxides and lactones in oxidized

lipoproteins, imparting protection to LDL against oxidation [4, 6, 25, 26, 28, 29]. LDL oxidation could be related to LDL-C levels [25] and/or to obesity [32, 33]. Weight loss and caloric restriction diet induces the prominent reduction of the LDL oxidation [32, 34]. Our study demonstrated a weak correlation between the decrease levels of BMI and those of LDL-C ($r = 0.288$, $p > 0.05$ [data not shown in the result section]), as well as an independently significant correlation of the decrease levels of PON-1 to those of BMI or LDL-C (shown in Table 2). Such a weak correlation between obesity and LDL-C (a quantity measure of LDL), relative to the measures of LDL oxidation (a concept of quality of LDL), has been noted previously [32, 33]. Thus, our results on the relationship between PON-1 and LDL-C might be explained, a priori, as a consequence of less need for LDL protection by the LDL-C concentration decrease and weight loss following a LCD intervention. Importantly, in the last years, the re-evaluation of HDL physiology stresses its pro- or anti-inflammatory functions according to different circumstances and its pleiotropic functional activities [35, 36]. Hence, the decrease in PON-1 as well as HDL-C in response to LCD may not be detrimental but a physiologically appropriate or adaptive phenomenon. Whether this is due to adjustments in the expression of PON-1 or to re-distribution of HDL particles needs to be addressed in further studies.

As a study limitation, we examined a restricted number of women only. More studies on a larger scale of subjects, including men, and other ethnic populations (may have different genetic factors) are needed to establish our results [7, 13]. Also, studies evaluating PON-1 mass changes are warranted. Even though we did not measure actual PON-1 mass, the fact that all three PON-1 activities measured are reduced in a similar manner suggests that the changes are likely due to changes in PON-1 concentrations. Since the correlation with changes in HDL-C is not so strong, this may indicate lower amounts of PON-1 per unit HDL particle.

Conclusions

In a cohort of healthy overweight/obese women, we showed for the first time a reduced PON-1 lactonase activity (as well as its mono- and tri-esterase activities) during a LCD intervention on weight loss, with a significant correlation with the reduction of LDL-C. The present results on the relationship between PON-1 and LDL-C may be pointing to the PON-1 activity level needed to protect LDL from oxidation, as an adaptive response to the LDL-C decrease and weight loss by the diet treatment. Moreover, the apparently paradoxical changes in HDL-C and PON-1 lactonase activity seen in the diet on weight loss would emphasize the need for the clinical laboratory to explore lipoprotein metabolisms beyond the simple static HDL-C

measurement. Further research is needed to confirm our theory.

Acknowledgments

This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of education, Culture, Sports, Science, and Technology of Japan (K.K. and S.K.). The authors are grateful to Prof. Dan Tawfik, Department of Biological Chemistry, Weizmann Institute of Science, Rehovot, Israel for kindly providing us with TBBL reagent, Dr. Jacques Toyal-Dope for critical reading of the manuscript and to Ms Claire Trias for editorial assistance.

References

- [1] Haffner, S.M.: Relationship of metabolic risk factors and development of cardiovascular disease and diabetes. *Obesity*, **14**, S121–S127, 2006.
- [2] Ferretti, G., Bacchetti, T., Moroni, C., Savino, S., Liuzzi, A., Balzola, F., and Bicchiega, V.: Paraoxonase activity in high-density lipoproteins: a comparison between healthy and obese females. *J. Clin. Endocrinol. Metab.*, **90**, 1728–1733, 2005.
- [3] Liu, Y., Mackness, B., and Mackness, M.: Comparison of the ability of paraoxonases 1 and 3 to attenuate the *in vitro* oxidation of low-density lipoprotein and reduce macrophage oxidative stress. *Free Radic. Biol. Med.*, **45**, 743–748, 2008.
- [4] Durrington, P.N., Mackness, B., and Mackness, M.I.: Paraoxonase and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.*, **21**, 473–480, 2001.
- [5] Kim, N.S., Kang, K., Cha, M.H., Kang, B.J., Moon, J., Kang, B.K., Yu, B.C., Kim, Y.S., Choi, S.M., and Bang, O.S.: Decreased paraoxonase-1 activity is a risk factor for ischemic stroke in Koreans. *Biochem. Biophys. Res. Commun.*, **364**, 157–162, 2007.
- [6] Sharma, R., Singh, B., and Mahajan, M.: PON-1 activity is inversely related to LDL apoB carbonyl content in patients with coronary artery disease. *Kaohsiung J. Med. Sci.*, **23**, 225–231, 2007.
- [7] Bhattacharyya, T., Nicholls, S.J., Topol, E.J., Zhang, R., Yang, X., Schmitt, D., Fu, X., Shao, M., Brennan, D.M., Ellis, S.G., Brennan, M.L., Allayee, H., Lusis, A.J., and Hazen, S.L.: Relationship of paraoxonase 1 (PON1) gene polymorphisms and functional activity with systemic oxidative stress and cardiovascular risk. *JAMA*, **299**, 1265–1276, 2008.
- [8] Hill, J.O. and Wyatt, H.: Outpatient management of obesity: a primary care perspective. *Obes. Res.*, **2**, S124–S130, 2002.
- [9] Berkel, L.A., Poston, W.S., Reeves, R.S., and Foreyt, J.P.: Behavioral interventions for obesity. *J. Am. Diet. Assoc.*, **105**, S35–S43, 2005.
- [10] Uzun, H., Zengin, K., Taskin, M., Aydin, S., Simsek, G., and Dariyerli, N.: Changes in leptin, plasminogen activator factor and oxidative stress in morbidly obese patients following open and laparoscopic Swedish adjustable gastric banding.

- Obes. Surg.*, **14**, 659–665, 2004.
- [11] Audikovszky, M., Pados, G., Seres, I., Harangi, M., Fülöp, P., Katona, E., Illyés, L., Winkler, G., Katona, E.M., and Paragh, G.: Orlistat increases serum paraoxonase activity in obese patients. *Nutr. Metab. Cardiovasc. Dis.*, **17**, 268–273, 2007.
- [12] Rector, R.S., Warner, S.O., Liu, Y., Hinton, P.S., Sun, G.Y., Cox, R.H., Stump, C.S., Laughlin, M.H., Dellsperger, K.C., and Thomas, T.R.: Exercise and diet induced weight loss improves measures of oxidative stress and insulin sensitivity in adults with characteristics of the metabolic syndrome. *Am. J. Physiol. Endocrinol. Metab.*, **293**, E500–E506, 2007.
- [13] Thompson, P.D., Jeffery, R.W., Wing, R.R., and Wood, P.D.: Unexpected decrease in plasma high density lipoprotein cholesterol with weight loss. *Am. J. Clin. Nutr.*, **32**, 2016–2021, 1979.
- [14] Shoji, T., Nishizawa, Y., Koyama, H., Hagiwara, S., Aratani, H., Izumotani-Sasao, K., Kishimoto, H., Tanishita, H., and Morii, H.: Lipoprotein metabolism in normolipidemic obese women during very low calorie diet: changes in high density lipoprotein. *J. Nutr. Sci. Vitaminol.*, **37**, S57–S64, 1991.
- [15] Cominacini, L., Garbin, U., Davoli, A., Campagnola, M., De Santis, A., Pasini, C., Pastorino, A.M., and Bosello, O.: Influence of weight reduction on plasma high-density-lipoprotein cholesterol concentrations in severe obesity: interrelationships with plasma insulin levels. *Ann. Nutr. Metab.*, **35**, 339–346, 1991.
- [16] Barnard, R.J.: Effects of life-style modification on serum lipids. *Arch. Intern. Med.*, **151**, 1389–1394, 1991.
- [17] Tzotzas, T., Samara, M., Constantinidis, T., Tziomalos, K., and Krassas, G.: Short-term administration of orlistat reduced daytime triglyceridemia in obese women with the metabolic syndrome. *Angiology*, **58**, 26–33, 2007.
- [18] Brinton, E.A., Eisenberg, S., and Breslow, J.L.: A low-fat diet decreases high density lipoprotein (HDL) cholesterol levels by decreasing HDL apolipoprotein transport rates. *J. Clin. Invest.*, **85**, 144–151, 1990.
- [19] Katznel, L., Coon, P.J., Dengel, J., and Goldberg, A.P.: Effects of an American Heart Association step I diet and weight loss on lipoprotein lipid levels in obese men with silent myocardial ischemia and reduced high-density lipoprotein cholesterol. *Metabolism*, **44**, 307–314, 1995.
- [20] Mosinger, B.J.: Human low-density lipoproteins: oxidative modification and its relation to age, gender, menopausal status and cholesterol concentrations. *Eur. J. Clin. Chem. Clin. Biochem.*, **35**, 207–214, 1997.
- [21] Nieman, D.C., Haig, J.L., Fairchild, K.S., De Guia, E.D., Dizon, G.P., and Register, U.D.: Reducing-diet and exercise-training effects on serum lipids and lipoproteins in mildly obese women. *Am. J. Clin. Nutr.*, **52**, 640–645, 1990.
- [22] Wood, P.D.: Physical activity, diet, and health: independent and interactive effects. *Med. Sci. Sports Exerc.*, **26**, 838–843, 1994.
- [23] Yamanouchi, K., Shinozaki, T., Chikada, K., Nishikawa, T., Ito, K., Shimizu, S., Ozawa, N., Suzuki, Y., Maeno, H., Kato, K., Oshida, Y., and Sato, Y.: Daily walking combined with diet therapy is a useful means for obese NIDDM patients not only to reduce body weight but also to improve insulin sensitivity. *Diabetes Care*, **18**, 775–778, 1995.
- [24] Nikolaidis, M.G. and Mougios, V.: Effects of exercise on the fatty-acid composition of blood and tissue lipids. *Sports Med.*, **34**, 1051–1076, 2004.
- [25] Khersonsky, O. and Tawfik, D.S.: The histidine 115-histidine 134 dyad mediates the lactonase activity of mammalian serum paraoxonases. *J. Biol. Chem.*, **281**, 7649–7656, 2006.
- [26] Khersonsky, O. and Tawfik, D.S.: Chromogenic and fluorogenic assays for the lactonase activity of serum paraoxonases. *Chembiochem*, **7**, 49–53, 2006.
- [27] Zimmerman, J., Kaufmann, N.A., Fainaru, M., Eisenberg, S., Oschry, Y., Friedlander, Y., and Stein, Y.: Effect of weight loss in moderate obesity on plasma lipoprotein and apolipoprotein levels and on high density lipoprotein composition. *Atherosclerosis*, **4**, 115–123, 1984.
- [28] Mackness, M.I., Durrington, P.N., and Mackness, B.: The role of paraoxonase I activity in cardiovascular disease: potential for therapeutic intervention. *Am. J. Cardiovasc. Drugs*, **4**, 211–217, 2004.
- [29] Aviram, M. and Rosenblat, M.: Paraoxonases and cardiovascular diseases: pharmacological and nutritional influences. *Curr. Opin. Lipidol.*, **16**, 393–399, 2005.
- [30] Rantala, M., Silaste, M.L., Tuominen, A., Kaikkonen, J., Salonen, J.T., Alftan, G., Aro, A., and Kesäniemi, Y.A.: Dietary modifications and gene polymorphisms alter serum paraoxonase activity in healthy women. *J. Nutr.*, **132**, 3012–3017, 2002.
- [31] Domagala, T.B., Lacinski, M., Trzeciak, W.H., Mackness, B., Mackness, M.I., and Jakubowski, H.: The correlation of homocysteine-thiolactonase activity of the paraoxonase (PON1) protein with coronary heart disease status. *Cell Mol. Biol.*, **52**, 4–10, 2006.
- [32] Vasankari, T., Fogelholm, M., Kukkonen-Harjula, K., Nenonen, A., Kujala, U., Oja, P., Vuori, I., Pasanen, P., Neuvonen, K., and Ahotupa, M.: Reduced oxidized low-density lipoprotein after weight reduction in obese premenopausal women. *Int. J. Obes. Relat. Metab. Disord.*, **25**, 205–211, 2001.
- [33] Holvoet, P., Lee, D.H., Steffes, M., Gross, M., and Jacobs, D.R. Jr.: Association between circulating oxidized low-density lipoprotein and incidence of the metabolic syndrome. *JAMA*, **299**, 2287–2293, 2008.
- [34] Shin, M.J., Hyun, Y.J., Kim, O.Y., Kim, J.Y., Jang, Y., and Lee, J.H.: Weight loss effect on inflammation and LDL oxidation in metabolically healthy but obese (MHO) individuals: low inflammation and LDL oxidation in MHO women. *Int. J. Obes.*, **30**, 1529–1534, 2006.
- [35] Roberts, C.K., Ng, C., Hama, S., Eliseo, A.J., and Barnard, R.J.: Effect of a short-term diet and exercise intervention on inflammatory/anti-inflammatory properties of HDL in overweight/obese men with cardiovascular risk factors. *J. Appl. Physiol.*, **101**, 1727–1732, 2006.
- [36] Navab, M., Reddy, S., Van Lenten, B.J., Anantharamaiah, G.M., and Fogelman, A.M.: The role of dysfunctional HDL in atherosclerosis. *J. Lipid Res.*, **50**, S145–S149, 2009.