Lipoprotein Metabolism in Normolipidemic Obese Women during Very Low Calorie Diet: Changes in High Density Lipoprotein

Tetsuo Shoji*, Yoshiki Nishizawa, Hidenori Koyama, Satoshi Hagiwara, Hideyuki Aratani, Kyoko Izumotani-Sasao, Hiroshi Kishimoto, Hitoshi Tanishita, and Hirotoshi Morii

Second Department of Internal Medicine, Osaka City University Medical School, 1-5-7, Asahi-machi, Abeno-ku, Osaka 545, Japan

Summary We examined changes in high density lipoprotein (HDL) metabolism during a very low calorie diet weight reduction program in 6 massively obese normolipidemic women. The diet protocol consisted of a 1st low calorie diet (LCD; 1440, 1280, and 880 kcal daily for 1 week, each, in succession), a 1st very low calorie diet (VLCD; 420 kcal daily for 4 weeks, using Optifast 70), intermission (880 kcal daily for 1 week), the 2nd VLCD (4 weeks) and the 2nd LCD (880 and 1280–1440 kcal daily for 1 week, each). Mean body weight reduction was 18.9 kg. HDL-cholesterol, more specifically HDL2-cholesterol, reduced transiently during the 1st VLCD, intermission, and 2nd VLCD periods, and tended to increase in the 2nd LCD. Apolipoprotein (apo) A-I showed a similar change to HDL-cholesterol. However, apo A-II decreased persistently throughout the weight reduction program, and the apo A-I/apo A-II ratio increased significantly in the later part of the program. Serum triglyceride, apo B, and lipoprotein lipase (LPL) activity in post-heparin plasma did not change. These data suggest that the observed decrease in HDL-cholesterol was not due to a reduction in very low density lipoprotein-derived HDL production or to an LPL deficiency, but was consistent with reduction in chylomicron-derived HDL formation following dietary fat restriction.

Key Words obesity, very low calorie diet, high density lipoprotein, apolipoprotein, lipoprotein lipase

Obesity is often associated with lipoprotein abnormalities, including increased serum triglyceride, with or without high cholesterol levels, and lowered high density lipoprotein (HDL) cholesterol. Although reduced HDL-cholesterol level is an independent risk factor for atherosclerosis (1), the underlying mechanism of low HDL-cholesterol in obesity is still unclear; several possibilities have been proposed. Firstly, the reciprocal relationship between serum triglyceride and HDL-cholesterol (2) may indicate reduced production of HDL precursor during the hydrolysis of triglyceride-rich lipoproteins, chylomicron and

*To whom all correspondence should be addressed.
very low density lipoprotein (VLDL), by the action of lipoprotein lipase (LPL) (3). Otherwise, the relationship may result from enhanced lipid transfer reaction between HDL and triglyceride-rich lipoproteins, mediated by cholesteryl ester transfer protein (CETP) in the presence of hypertriglyceridemia (4), which is often seen in obese subjects. Secondly, adipose cells possess binding sites for HDL, and these cells incorporate HDL (5). Therefore, increased fat mass may result in the increased uptake of HDL and subsequent reduction in plasma HDL-cholesterol levels (6). Thirdly, diets which maintain obesity may relate directly to low HDL levels. Obese patients consume high fat and/or high carbohydrate diets. Furthermore, physical activity is an important factor in determining HDL-cholesterol. A very low calorie diet (VLCD), providing extremely low fat and carbohydrate with adequate protein, is a rapid and effective way of selective reduction of fat mass in massive obesity (6). Although lipid and lipoprotein metabolism can be altered in patients on VLCD, little is known about alterations in HDL metabolism. In the present study, we examined HDL metabolism during a VLCD weight reduction program in 6 massively obese normolipidemic women.

**PATIENTS AND METHODS**

*Patients.* The subjects of this study were 6 consecutive massively obese women with serum cholesterol <220 mg/dl and triglyceride <150 mg/dl. The diagnosis of simple obesity was made by careful ruling-out of secondary obesity due to thyroid and adrenal dysfunction, polycystic ovary, intracranial lesions, and congenital syndromes. Computed tomography of the abdomen revealed the predominance of subcutaneous fat accumulation over visceral fat in all cases. In the baseline study, mean (range) age, body weight, height, and body mass index were 39 (25–45) yr, 105 (69–156) kg, 154 (146–161) cm, and 44.3 (33.6–62.5) kg/m², respectively. Serum cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol were 189 (146–215), 84 (56–130) 42.6 (31.0–55.6) and 130 (65–142) mg/dl, respectively.

*Weight reduction protocol.* The diet protocol was similar to that used previously (7) and consisted of 5 parts; a 1st low calorie diet (LCD, 3 weeks), a 1st very low calorie diet (VLCD, 4 weeks), intermission (1 week), the 2nd VLCD (4 weeks), and the 2nd LCD (2–3 weeks). In the 1st LCD, daily energy intake was reduced weekly from 1440 kcal to 1280 kcal, and finally to 880 kcal, with conventional meals which contained 60–80 g of protein, and 55, 45, and 27 g of fat, respectively. In the 1st and 2nd VLCD periods, the patients took 5 packs of Optifast 70 daily, which provided 70 g of protein, 2 g of fat, and 32 g of carbohydrate, with a total energy of 420 kcal. In the intermission period, the same 880 kcal meal used in the 1st LCD was given. In the 2nd LCD, daily energy intake was increased weekly from 880 kcal to 1280 or 1440 kcal with the same menu used in the 1st LCD period. Throughout the diet therapy, the physical activity of each patient was kept roughly constant; the patients walked 10,000–20,000 steps in the hospital and/or used a bicycle ergometer at an intensity of 60% of the anaerobic threshold. Figure 1 illustrates the diet protocol and changes in body composition in a representative case, showing selective reduction in fat mass, with lean body mass and bone mineral kept constant.

*J. Nutr. Sci. Vitaminol.*
Fig. 1. Diet protocol and a representative course of weight reduction. Body composition was measured by dual photon absorptiometry using a Norland (Wisconsin) bone densitometer model 2600 (7).

Analysis of serum lipids, lipoproteins, apolipoproteins, and lipoprotein-regulating enzymes. Fasting blood was collected before the 1st LCD (baseline) and at each end of the 5 segments of the diet protocol. Serum cholesterol and triglycerides were measured by enzymatic methods. HDL-cholesterol was measured in the supernatant after precipitating apolipoprotein B-containing particles, using dextran sulfate and Mg²⁺. LDL-cholesterol was calculated using the Friedewald formula (8). Apolipoproteins A-I, A-II, B, C-II, C-III, and E were measured by turbidoimmunoassay (ApoAuto, Daiichi, Osaka, Japan). Plasma lecithin:cholesterol acyltransferase (LCAT) activity was measured by an exogenous substrate method (Anasorv LCAT, Daiichi, Osaka, Japan). Lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) activities were measured in post-heparin plasma, obtained 10 min after the intravenous injection of heparin (30 IU/kg body weight), by methods described elsewhere (9). HDL2- and HDL3-cholesterol were measured in 4 of the 6 patients, by 1-step preparative ultracentrifugation using KBr; HDL3-cholesterol was measured as cholesterol concentration in the d>1.125 fraction. HDL2-cholesterol (1.063<d<1.125) was calculated as the difference in cholesterol concentrations between the d>1.063 and the d>1.125 fractions.

Statistics. Data were expressed as means±SE. Differences between repeated measurements were evaluated by 2-way analysis of variance (ANOVA) with Scheffe type multiple comparison. A p-value<0.05 was considered as significant.

RESULTS

Body weight Change. All of the 6 patients successfully reduced weight on the diet therapy. Mean total weight reduction was 18.9 kg (range 14.3–31.4 kg), which consisted
of 12.7 kg (range 9.6–16.0 kg) during the 1st LCD-VLCD period and 6.2 kg (range 1.2–16.4 kg) during the remaining parts.

Changes in serum lipids, apolipoproteins, and lipoprotein-regulating enzyme activity. Table 1 shows the changes in serum lipids, apolipoproteins, and lipoprotein-regulating enzyme activity. Total cholesterol was reduced transiently after the 1st VLCD. Triglyceride and LDL-cholesterol did not change significantly. HDL-cholesterol, measured by a precipitation method, was reduced during the 1st VLCD-intermission-2nd VLCD period, and tended to increase thereafter. There was no significant change in apo B, C-II, E, LPL, or HTGL levels.

Changes in HDL subfractions. As shown in Fig. 2, HDL-cholesterol change was confirmed by the ultracentrifugation method. HDL_{3}-cholesterol did not change significantly, whereas the HDL_{2} fraction was reduced during the 1st VLCD, intermission, and the 2nd VLCD periods. The ratio of HDL_{2}/HDL_{3}-cholesterol also decreased in these periods. HDL_{2}-cholesterol and the HDL_{2}/HDL_{3}-cholesterol ratio increased during the 2nd LCD period.

Changes in Apolipoproteins A-I and A-II. Figure 3 shows the changes in apo A-I and apo A-II, both being the major apolipoproteins of HDL particles. Corresponding to

Fig. 2. HDL subfractions during weight reduction program. Cholesterol in HDL_{2} (1.063<d<1.125) and HDL_{3} (d>1.125) was measured by preparative ultracentrifugation in 4 of the 6 patients. *p<0.05 vs. baseline. Change in HDL_{2}/HDL_{3} ratio was significant (p<0.02) by 2-way ANOVA. Mean±SE.
Table 1. Changes in serum lipids, lipoproteins, apolipoproteins, and lipoprotein-regulating enzyme levels during the weight reduction program.

|                      | Baseline | 1st LCD | 1st VLCD | Intermission | 2nd VLCD | 2nd LCD | ANOVA    |
|----------------------|----------|---------|----------|--------------|----------|---------|----------|
| TC (mg/dl)           | 189±10   | 164±11  | 153±7*   | 172±15       | 168±11   | 171±16  | p=0.021  |
| TG (mg/dl)           | 84±11    | 70±8    | 84±10    | 91±10        | 84±9     | 85±7    | NS       |
| HDL-C (mg/dl)        | 42.6±4.1 | 37.0±2.0| 31.4±2.4 | 34.3±1.9     | 33.7±2.2 | 36.9±2.1| p=0.0017 |
| LDL-C (mg/dl)        | 130±10   | 112±11  | 105±7    | 119±14       | 117±11   | 117±15  | NS       |
| LCL-C/HDL-C ratio    | 3.24±0.48| 3.11±0.40| 3.45±0.38| 3.50±0.42    | 3.56±0.46| 3.32±0.66| NS       |
| apo A-I (mg/dl)      | 107±7    | 94±3    | 81±3*    | 90±2*        | 84±3*    | 96±2    | p=0.0001 |
| apo A-II (mg/dl)     | 28.0±2.7 | 22.8±1.6*| 21.1±1.0*| 21.2±1.5*    | 18.2±1.7*| 17.8±1.5*| p=0.0001 |
| apo B (mg/dl)        | 89±9     | 81±7    | 79±4     | 81±7         | 82±6     | 81±8    | NS       |
| apo C-II (mg/dl)     | 2.8±0.3  | 2.6±0.3 | 3.0±0.4  | 3.0±0.4      | 2.7±0.4  | 2.6±0.3 | NS       |
| apo C-III (mg/dl)    | 5.4±1.0  | 4.0±0.7 | 4.2±0.8  | 5.1±0.6      | 4.0±0.7  | 5.0±0.4 | p=0.0293 |
| apo E (mg/dl)        | 5.7±0.7  | 4.3±0.5 | 4.4±0.7  | 4.9±0.4      | 4.3±0.6  | 4.7±0.4 | NS       |
| apo B/A-I ratio      | 0.84±0.09| 0.87±0.09| 0.98±0.05| 0.90±0.07    | 0.99±0.09| 0.85±0.10| NS       |
| apo C-II/C-III ratio | 0.59±0.09| 0.75±0.14| 0.80±0.10| 0.61±0.05    | 0.73±0.09| 0.52±0.05| p=0.031  |
| apo C-III/E ratio    | 1.02±0.18| 0.90±0.11| 0.91±0.07| 1.03±0.08    | 0.92±0.08| 1.08±0.08| NS       |
| LPL(µ mol/h/ml)      | 3.36±0.60| 3.32±0.62| 2.84±0.57| 2.84±0.46    | 2.84±0.35| 2.23±0.22| NS       |
| HTGL(µ mol/h/ml)     | 9.86±2.04| 7.88±0.95| 6.46±1.92| 7.16±1.81    | 6.85±1.67| 5.94±0.58| NS       |
| LCAT (nmol/h/ml)     | 390±38   | 407±20  | 286±14   | 315±33       | 242±32*  | 253±21  | p=0.0035 |

TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; apo, apolipoprotein; LPL, lipoprotein lipase; HTGL, hepatic triglyceride lipase; LCAT, lecithin: cholesterol acyltransferase. Mean±SE, *p < 0.05 vs. baseline. Normal ranges of the enzymes for female: LPL, 1.55–2.63 (µ mol/h/ml); HTGL, 3.65–12.28 (µ mol/h/ml); LCAT, 235–550(nmol/h/ml).
Fig. 3. Changes in apolipoproteins A-I and A-II. Apolipoproteins A-I and A-II were measured by turbidoimmunoassay. Note the difference between the responses of apo A-I and A-II in the later part of the weight reduction protocol. *p<0.05 vs. baseline. Mean±SE.

the decrease in HDL-cholesterol, apo A-I and A-II decreased during the 1st VLCD, intermission, and the 2nd VLCD periods. However, apo A-I and apo A-II showed different responses after that; apo A-I increased, whereas apo A-II decreased further. Therefore, there was a significant and persistent increase in the apo A-I/apo A-II ratio in the later part of the diet therapy.

DISCUSSION

In the present study, we observed several changes in lipid and lipoprotein metabolism in normolipidemic obese women who were treated with a very low calorie diet. The findings were: transient decreases in serum cholesterol, HDL-cholesterol, and apo A-I, a persistent reduction in apo A-II, and no significant change in serum triglyceride, apo B, LPL, or HTGL. Among these, alteration in HDL metabolism was of interest.

Obesity is often associated with low HDL-cholesterol (9), which is possibly linked to the risk of atherosclerosis in these patients. The underlying mechanism may consist of increased degradation and/or decreased production of HDL particles. Adipose cells have binding sites for HDL and they incorporate this class of lipoprotein (5). Thus,

J. Nutr. Sci. Vitaminol.
accumulation of body fat may result in an increased uptake of HDL particles from circulation and a subsequent reduction in plasma HDL concentration. Weight reduction or decrease in body fat, could then bring about an increased HDL level. In this study, however, HDL-cholesterol showed transient decreases and subsequent increases. This symmetrical change in HDL-cholesterol occurred in parallel to body weight reduction, but in the protocol itself. Therefore, in this relatively short-term study, HDL metabolism in the patients seemed to be affected largely by dietary modulation.

HDL has 2 major sources of origin; one is the direct secretion of HDL precursor from the liver and intestine and the other is surface remnants of triglyceride-rich lipoproteins generated during the delipidation process mediated by lipoprotein lipase (10). These HDL precursors, called nascent HDL, have a discoidal shape; they become mature spherical HDL3, and finally HDL2, as a result of the action of LCAT. Although both fat and carbohydrate were restricted in the diet in this study, neither serum triglyceride or apo B changed significantly, suggesting that very low density lipoprotein (VLDL) production was not changed following the LCD and VLCD periods. Increased mobilization of fatty acids from adipose tissues to the liver could compensate for the depletion of substrate for triglyceride synthesis. In contrast, since Optifast 70 contained only 2 g of fat, chylomicron production must have been critically reduced during the VLCD period. In a situation like this, chylomicron-derived HDL particles could decrease, while VLDL-derived HDL could remain constant. LPL activity in post-heparin plasma showed little change and maintained high normal levels, suggesting that the decreased HDL level was not due to LPL deficiency. Although changes in the direct secretion of HDL precursors by the liver and intestine were possible, it is likely that the transient decrease in HDL-cholesterol and apo A-I was due to the restriction of fat intake and subsequent decrease in chylomicron-derived HDL production. Similar observations have been made by other works (1–13). Brinton et al. reported HDL-cholesterol reduction during an “anti-atherogenic diet” with a low fat content and high P/S ratio (13). They studied the in vivo kinetics of apo A-I and found that reduced HDL-cholesterol was associated with a decreased transport (production) rate but not with the fractional catabolic rate. These findings, which indicate that a low fat diet resulted in reduced HDL formation, support our speculation.

Epidemiological studies have demonstrated the protective role of HDL for coronary heart disease (1), whereas an “anti-atherogenic” diet seems to decrease HDL-cholesterol, as shown by us and others (11–13). What causes this apparent discrepancy? Brinton found, in the study cited above, that variation in HDL-cholesterol between subjects correlated with variations in the fractional catabolic rate, but not with the transport rate, of apo A-I (14). Thus, it seems important to determine which mechanism is responsible for the reduced HDL-cholesterol level, decreased synthesis or increased catabolism of HDL.

Apo A-II decreased persistently during our diet program, although reductions in apo A-I and HDL-cholesterol were transient. The apo A-I/apo A-II ratio increased in the later part of the protocol, indicating the compositional changes in the HDL fraction. Immunoaffinity chromatography, using antibodies against apo A-I and A-II, revealed the presence of HDL subpopulations, called LpA-I and LpA-I:A-II. LpA-I contains apo A-I but no apo A-II, and LpA-I:A-II contains both apo A-I and A-II. Thus, the observed
increase in the apo A-I/apo A-II ratio may indicate increased LpA-I and decreased LpA-I:A-II subpopulations. A previous study showed the anti-atherogenic effect of HDL in the LpA-I subpopulation only (15). Therefore, the change in HDL metabolism found in this study may be a favorable one.

REFERENCES

1) Castelli, W. P., Garrison, R. J., Wilson, P. W. F., Abbot, R. D., Kalousdian, S., and Kannel, W. B. (1986): Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham study. J. Am. Med. Assoc., 256, 2835–2838.

2) Miller, G. L., and Miller, N. E. (1975): Plasma-high-density-lipoprotein concentration and development of ischemic heart disease. Lancet, 1, 16–19.

3) Nikkila, E. A. (1978): Metabolic regulation of plasma high density lipoprotein concentration. Eur. J. Clin. Invest., 8, 111-113.

4) Eisenberg, S. (1985): Preferential enrichment of large-sized very low density lipoprotein with transferred cholesteryl esters. J. Lipid. Res., 26, 487–494.

5) Fong, B. S., Rodrigues, P. O., Salter, A. M., Yip, B. P., Despres, J.-P., Angel, A., and Gregg, R. E. (1985): Characterization of high density lipoprotein binding to human adipocyte plasma membranes. J. Clin. Invest., 75, 1804–1812.

6) Lewis, B. (1978): Effects of diets and drugs, in High Density Lipoprotein in Atherosclerosis. Elsevier, Amsterdam, pp.143–148

7) Koyama, H., Nishizawa, Y., Yamashita, N., Furumitsu, Y., Hagiwara, S., Ochi, H., and Morii, H. (1990): Measurement of composition changes using dual photon absorptiometry in obese patients undergoing semistarvation. Metabolism, 39, 302–306.

8) Friedewald, W. T., Levy, R. I., and Fredrickson, D. S. (1962): Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem., 18, 499–502.

9) Shoji, T., Nishizawa, Y., Nishitani, H., Yamakawa, M., and Morii, H. (1991): Roles of hypoalbuminemia and lipoprotein lipase on hyperlipoproteinemia in continuous ambulatory peritoneal dialysis. Metabolism, 40, 1002–1008.

10) Eisenberg, S. (1984): High density lipoprotein metabolism. J. Lipid. Res., 25, 1017–1058.

11) Thompson, P. D., Jeffery, R. W., Wing, R. R., and Wood, P. D. (1979): Unexpected decrease in plasma high density lipoprotein cholesterol with weight reduction. Am J. Clin. Nutr., 32, 2016–2021.

12) Wechsler J. G., Hutt, V., Wenzel, H., Kloer, H.-U., and Ditschunet, H. (1981): Lipid and lipoproteins during a very-low-calorie diet. Int. J. Obesity, 5, 325–331.

13) Tokunaga, K., Ishikawa, K., and Matsuzawa, Y. (1982): Lipids and lipoproteins during a very low calorie diet. Int. J. Obesity, 6, 416.

14) Brinton, E. A., Eisenberg, S., and Breslow, J. L. (1991): Increased apo A-I and apo A-II fractional catabolic rate in patients with low high density lipoprotein-cholesterol levels with or without hypertriglyceridemia. J. Clin. Invest., 87, 536–544.

15) Puchois, P., Kandoussi, A., Fievet, P., Fournie, J. L., Bertrand, M., Koren, E., and Fruchart, J. C. (1987): Apolipoprotein A-I containing lipoproteins in coronary artery disease. Atherosclerosis, 68, 35–40.