

# Beneficial Effect on Average Lipid Levels From Energy Restriction and Fat Loss in Obese Individuals With or Without Type 2 Diabetes

TANIA P. MARKOVIC, FRACP  
 LESLEY V. CAMPBELL, FRACP  
 SANTHIRA BALASUBRAMANIAN, PHD  
 ARTHUR B. JENKINS, PHD

ADRIANA C. FLEURY, MNUEDIET  
 LEON A. SIMONS, MD  
 DONALD J. CHISHOLM, FRACP

**OBJECTIVE** — The risk of cardiovascular disease in type 2 diabetes is greater than is accounted for by conventional risk factors. We investigated whether energy restriction or modest fat loss improved the lipid profile in obese subjects with and without type 2 diabetes. The relationship of site of adipose tissue loss to lipid changes was also examined.

**RESEARCH DESIGN AND METHODS** — Lipid levels were measured in 18 subjects with normal glucose tolerance (NGT) ( $n = 9$ , BMI =  $31.5 \pm 0.8$  [SEM] kg/m<sup>2</sup>) or type 2 diabetes ( $n = 9$ , BMI =  $31.8 \pm 0.7$ ) before and on the 4th (d4) and 28th (d28) days of a hypocaloric formula diet. Body composition was assessed with dual energy X-ray absorptiometry on d0 and d28.

**RESULTS** — Mean daily energy intake during the diet was  $1,100 \pm 60$  kcal (33% protein, 38% carbohydrate, and 29% fat). Mean weight loss was  $6.2 \pm 0.4$  kg. Initial lipid profiles were similar in subjects with or without diabetes, and diabetes did not affect the responses. Dietary intervention resulted in early (d4) and late (d28) changes. Energy restriction (d4) reduced VLDL cholesterol and total triglyceride (TG) concentrations and increased LDL particle size. With fat loss (d28), there were falls in total LDL cholesterol (free and esterified components), LDL TG, and LDL apolipoprotein B (apoB) concentrations. Reduction in central abdominal fat (but not other body fat) was correlated with a less atherogenic lipid profile:  $\Delta$  abdominal fat versus  $\Delta$  LDL free cholesterol,  $r = 0.65$ ,  $P = 0.006$  and versus  $\Delta$  apoB,  $r = 0.64$ ,  $P = 0.008$ .

**CONCLUSIONS** — Even in obese subjects with an average lipid profile, modest weight loss reduces atherogenicity, independently of type 2 diabetes, and abdominal fat loss is specifically related to such improvements.

**A**therosclerotic vascular disease is a major problem in patients with type 2 diabetes, who often have low HDL cholesterol and hypertriglyceridemia (1) accompanied by qualitative abnormalities such as smaller LDL particle size (2) and increased LDL oxidizability (3). However, obese patients with type 2 diabetes with average lipid levels still have increased risk

of cardiovascular disease. There is evidence that obesity, particularly abdominal obesity, which is highly prevalent in type 2 diabetes, may be equally important with regard to cardiac risk as diabetes per se (4).

Weight loss is central to type 2 diabetes management, being associated with improvements in glycemic control, insulin sensitivity, and lipid profile (5). Similarly, weight

loss improves insulin sensitivity and lipid profiles in obese individuals with normal glucose tolerance (NGT) (6). How diet exerts these beneficial effects is unclear, with evidence suggesting that caloric restriction and weight loss have independent effects (7,8), but whether improvements are related to the site of adipose tissue loss is uncertain.

This study assessed early and late changes in lipid levels induced by energy restriction versus fat loss, respectively, in obese subjects with or without type 2 diabetes. We report the changes in lipid profile, the influence of diabetes, and how lipid changes relate to fat loss.

## RESEARCH DESIGN AND METHODS

### Subjects

From the Diabetes Clinic, St. Vincent's Hospital, Sydney and through advertisements, 18 mildly obese subjects (BMI =  $31.5 \pm 0.8$  kg/m<sup>2</sup>), 9 (women  $n = 4$ , men  $n = 5$ ) with NGT and no family history of diabetes and 9 (women  $n = 5$ , men  $n = 4$ ) with type 2 diabetes, matched for age and anthropometry, were recruited. These subjects are a group for which data on insulin sensitivity in the liver and peripheral tissues have been reported previously (9). NGT subjects were screened with a fasting plasma glucose (FPG) level. Two subjects with FPG  $\geq 5.5$  mmol/l had a formal oral glucose tolerance test confirming NGT (FPG  $< 6.1$ , 2-h plasma glucose [PG]  $< 7.8$  mmol/l) (10). Subjects with diabetes were treated with diet alone ( $n = 6$ ) or low doses of oral medication (metformin  $n = 1$ , gliclazide  $n = 2$ ); none had clinical evidence of other endocrine, cardiac, hepatic, or renal disease. All subjects consumed  $< 20$  g alcohol/day and were accepted only if a dietitian considered them well-motivated to lose weight and if they had not dieted for 6 months. Data from two NGT subjects were excluded from the turnover results because of technical difficulties during the clamps. One subject with diabetes has been excluded from all final analyses because of

From the Garvan Institute of Medical Research (T.P.M., L.V.C., A.C.F., D.J.C.) and the Lipid Research Department (S.B., L.A.S.), St. Vincent's Hospital, Sydney; and the Metabolic Research Centre and Department of Biomedical Science (A.B.J.), University of Wollongong, Wollongong, New South Wales, Australia.

Address correspondence and reprint requests to Dr. Tania P. Markovic, Garvan Institute of Medical Research, St. Vincent's Hospital, Darlinghurst, NSW 2010, Australia. E-mail: t.markovic@garvan.unsw.edu.au.

Received for publication 15 September 1997 and accepted in revised form 4 February 1998.

**Abbreviations:** apoB, apolipoprotein B; d0, day 0; d4, day 4; d28, day 28; DXA, dual energy X-ray absorptiometry; FPG, fasting plasma glucose; NGT, normal glucose tolerance; PG, plasma glucose; TG, triglyceride; WHR, waist-to-hip ratio.

Table 1—Baseline clinical characteristics

	NGT	Type 2 diabetes	P value
n	9	9	
Age (years)	48.4 ± 3.6	47.6 ± 4.8	0.89
Sex (M:F)	5:4	4:5	NS
BMI (kg/m <sup>2</sup> )	31.5 ± 0.8	31.8 ± 0.7	0.77
Waist (cm)	98.5 ± 3.9	98.1 ± 2.6	0.94
WHR	0.90 ± 0.03	0.87 ± 0.02	0.39
Skinfold (% fat)	33.5 ± 2.7	35.6 ± 1.7	0.52
Total fat DXA (%)	37.4 ± 3.5	39.8 ± 3.0	0.59
Abdominal fat DXA (%)	42.2 ± 2.2	43.6 ± 1.4	0.60
PG (mmol/l)	5.0 ± 0.2	7.4 ± 0.8	0.009
Insulin (mU/l)	7.8 ± 0.7	14.5 ± 1.3	0.0004
Fructosamine* (μmol/l)	207 ± 4	263 ± 20	0.01

Data are n or means ± SEM. \*Fructosamine reference interval <285 μmol/l.

recommencement of sulphonylurea therapy toward the end of the diet. A second diabetic subject was excluded from analyses involving changes in body composition because of technical problems during the final dual energy X-ray absorptiometry (DXA) scan. All subjects gave written informed consent; the study protocol was approved by the Research Ethics Committee of St. Vincent's Hospital.

### Experimental protocol

The following parameters were assessed before the diet commenced: usual dietary composition, weight, anthropometry, and body composition (with DXA). Hyperinsulinemic euglycemic clamp studies (9) were performed at baseline (day 0 [d0]), and on days 4 (d4) and 28 (d28) of the diet. Before each study, fasting lipid levels were determined. Body composition and anthropometry were reassessed at d28. Participants discontinued hypoglycemic therapy at least 1 week before the first clamp study (d0).

### Diet

Dietary composition was assessed by the dietitian from 4-day food records, as described previously (11). Subjects were requested not to alter their level of physical activity throughout. Each subject was supplied with a formula diet for 4 weeks (Nutri-Metics International, Sydney, Australia) with composition per 100 g as follows: protein = 37 g, fat = 2.3 g (polyunsaturated fat 9%, monounsaturated fat 30%, and saturated fat 61%), carbohydrate = 40 g, and energy content = 330 kcal. Each subject's diet was supplemented with essential fatty acids and minerals and was customized for each sub-

ject on the basis of body size, age, and energy intake to reduce intake by 1,000 kcal/day, as described in Jenkins et al. (11). Subjects were weighed weekly and completed daily food records throughout.

### Anthropometric measurements

The following measurements were made with the subject fasting by one observer (T.P.M.): weight, height, triplicate measures of skinfold thicknesses, and waist (narrowest diameter between the xiphoid process of the sternum and the iliac crest) and hip (widest diameter over the greater trochanters) circumferences. Total body fat (%) was estimated from skinfold thickness (12).

### Body composition

DXA (Lunar DPX; LUNAR, Madison, WI) was used to measure fat mass and lean tissue for the total body and three standard regions: trunk (chest, abdomen, and pelvis), arms, and legs. Central abdominal fat was estimated using a manually determined window as previously (9).

### Analytical measurements

PG, serum insulin, and fructosamine were measured as previously described (9). Cholesterol, triglycerides (TGs), and HDL cholesterol were measured as described in Simons et al. (13). EDTA plasma was separated into VLDL ( $d < 1.006$ ) and LDL ( $1.006 < d < 1.063$ ) by sequential ultracentrifugation (14). LDL apoB was measured by rate immunonephelometry (15), and free and esterified cholesterol were measured after separation on thin-layer chromatography (16). The susceptibility of LDL to copper-induced oxidation was

expressed as the lag time to commencement of oxidation under standard conditions (13). LDL particle size was measured by gradient gel electrophoresis using non-denaturing polyacrylamide gels (17).

### Statistics

Statistical analyses were performed using general purpose software (Statview; Abacus Concepts, Berkeley, CA). Baseline characteristics of the two groups were compared by unpaired *t* test. Anthropometric changes over the whole study (prediet vs. d28) were assessed using paired *t* test. The effects of energy restriction versus weight loss were assessed by *t* test: prediet versus d4 and d4 versus completion (d28). Possible differences between NGT and diabetic groups were investigated by analysis of variance of variable differences (d4 minus d0, d28 minus d4) against group. Since no significant group or interaction effects were found for lipid data, only time effects are reported, and data for the two groups have been combined. Association among variables was examined using correlation/regression analysis. Possible group effects (NGT vs. diabetes) were investigated using analysis of covariance. Since no group effect was found for any situation analyzed, only results of simple univariate analysis are presented. Unless otherwise stated, all results are expressed as means ± SEM.

## RESULTS

### Clinical characteristics

The baseline characteristics of the subjects are listed in Tables 1 and 2. With the groups matched for age, BMI, waist circumference, waist-to-hip ratio (WHR), and total and regional body fat, the presence of diabetes did not affect the lipid profiles, both groups having average standard lipid levels.

### Dietary composition changes

Both groups had a similar dietary composition. There was a significant reduction in intake of all macronutrients in absolute terms, with ~50% reduction of energy intake (prediet vs. during diet: 2,300 ± 170 vs. 1,100 ± 60 kcal,  $P = 0.0001$ , range: 520–2,550 kcal and percent reduction range: 25–75%). The percentage of energy from carbohydrate did not alter (prediet vs. during diet: 38.4 ± 1.8 vs. 38.3 ± 0.7% total energy intake), but that from protein increased (19.2 ± 0.7 vs. 32.6 ± 0.8%). Total fat (prediet vs. during diet: 35.7 ± 1.2 vs. 28.8 ± 0.8%), alcohol (6.8 ± 2.1 vs. 1.3

**Table 2—Baseline lipid variables**

	NGT	Type 2 diabetes	P value
<i>n</i>	9	9	
Total cholesterol (mmol/l)	4.86 ± 0.24	5.07 ± 0.26	0.55
VLDL cholesterol (mmol/l)	0.46 ± 0.06	0.51 ± 0.06	0.58
LDL cholesterol (mmol/l)	2.47 ± 0.20	2.51 ± 0.12	0.85
HDL cholesterol (mmol/l)	1.32 ± 0.1	1.12 ± 0.09	0.15
Total TG (mmol/l)	1.02 ± 0.09	1.19 ± 0.11	0.26
LDL apoB (g/l)	0.73 ± 0.06	0.86 ± 0.07	0.15
Particle size (Å)	255 ± 3	256 ± 4	0.77
LDL oxidizability (min)	54.9 ± 3.2	58.2 ± 3.6	0.50
LDL free cholesterol (mmol/l)	0.63 ± 0.04	0.63 ± 0.06	0.93
LDL esterified cholesterol (mmol/l)	1.73 ± 0.17	1.91 ± 0.10	0.37
LDL TG (mmol/l)	0.23 ± 0.06	0.27 ± 0.06	0.61

Data are *n* or means ± SEM.

± 0.8%), and fiber decreased (26 ± 2 vs. 9 ± 1 g), and there were changes in the fatty acid composition of the diet (prediet vs. during diet: polyunsaturated fats = 5.9 ± 0.4 vs. 10.1 ± 0.5%, monounsaturated fats = 13.8 ± 0.6 vs. 11.9 ± 0.4%, and saturated fats = 16.1 ± 0.6 vs. 6.0 ± 0.4%).

### Anthropometric changes

There was a mean 1.8 ± 0.3 kg (1.6 ± 0.2% loss of initial weight) and 6.2 ± 0.4 kg (6.5 ± 0.4%) weight loss after d4 and d28, respectively (groups behaved similarly). Notably, a significantly greater proportion of fat was lost from the abdominal region compared with total body fat loss (Δ abdominal fat vs. Δ total body fat: % decrease = 14 ± 2 vs. 8 ± 1%, *P* = 0.004) (Table 3).

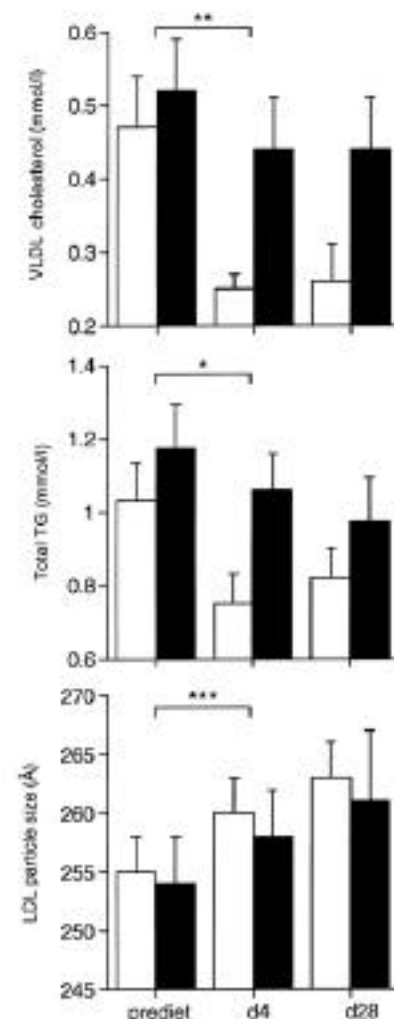
### Lipid changes

By d4, significant changes were evident in total TG, LDL particle size, and VLDL cholesterol levels (Fig. 1). Significant decreases in total and LDL cholesterol (free and esterified components) and LDL TG were

apparent only after fat loss at d28 (Fig. 2). At d28, there was also a significant fall in LDL apoB (Fig. 2), which was not measured at d4. There were no significant changes in HDL cholesterol or LDL oxidation susceptibility over the study (data not shown). Neither early nor late lipid changes related to the reduction in total energy intake, changes in any macronutrients, or fatty acid composition of the diet.

### Lipid and body compositional changes

At d0, there was a significant association between abdominal fat and total TGs (*r* = 0.45, *P* = 0.05) and esterified LDL cholesterol (*r* = 0.49, *P* = 0.04), but lipid levels were not related to total or any other regional fat mass. At d28, loss of abdominal fat was the only change in fat depots that related significantly to changes in the lipid profile, being strongly associated with the reduction in LDL free cholesterol (*r* = 0.65, *P* = 0.006) and LDL apoB (*r* = 0.64, *P* = 0.008) (Fig. 3); both relationships were independent of total



**Figure 1**—Lipids showing changes at d4: lipid levels prediet and at d4 and d28 (NGT subjects [□], *n* = 9 and type 2 diabetes [■], *n* = 8). \**P* < 0.01; \*\**P* < 0.005; and \*\*\**P* < 0.0005 vs. prediet levels. Since there are no group effects on this data, *P* values refer to a combined analysis of data from both groups.

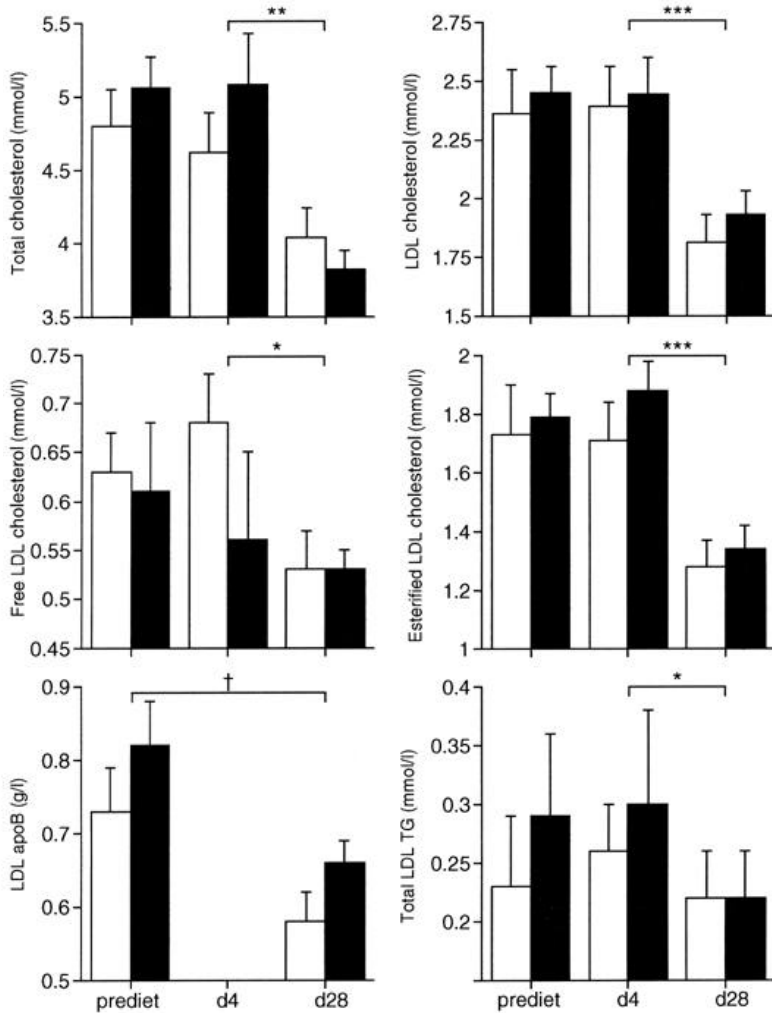
fat change in multiple regression (*P* = 0.007 and *P* = 0.01 for Δ LDL free cholesterol and Δ LDL apoB, respectively). Neither changes in waist circumference nor WHR were related to lipid changes.

**CONCLUSIONS**— Cardiovascular disease, the major cause of death in type 2 diabetes and obesity, is only partly related to abnormal lipid levels. Increasingly, the importance of qualitative abnormalities in the lipid profile, such as smaller, denser, and more easily oxidizable LDL particles (18) and higher LDL apoB levels, is being recognized, even when total cholesterol and subfractions are normal (19). In this study, obese subjects with diabetes had

**Table 3—Anthropometric changes**

	Prediet	Postdiet (d28)	P value
Weight (kg)	95.9 ± 2.7	89.9 ± 2.6	0.0001
BMI (kg/m <sup>2</sup> )	31.7 ± 0.5	29.6 ± 0.5	0.0001
Waist circumference (cm)	98.3 ± 2.3	92.6 ± 2.1	0.0001
WHR	0.88 ± 0.02	0.86 ± 0.02	0.002
Skinfold thickness (% fat)	34.6 ± 1.6	33.0 ± 1.6	0.0003
Total fat DXA (%)	38.6 ± 2.2	37.8 ± 2.3	0.03
Abdominal fat DXA (%)	42.9 ± 1.3	40.9 ± 1.6	0.01
Abdominal fat DXA (g)	2,700 ± 100	2,400 ± 100	0.0001

Data are means ± SEM. Combined data; similar changes were observed in both groups (NGT, *n* = 9 and type 2 diabetes, *n* = 9). Group data were compared by paired *t* test.



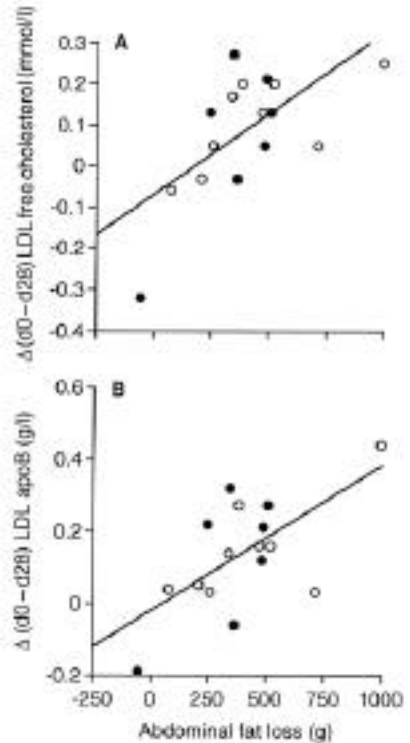
**Figure 2**—Lipids showing changes at d28: lipid levels prediet and at d4 and d28 (NGT subjects, n = 9 and type 2 diabetes, n = 8). \*P ≤ 0.05; \*\*P < 0.001; and \*\*\*P < 0.0005 vs. d4. †P = 0.001 vs. prediet. Since there are no group effects on this parameter, values refer to a combined analysis of data from both groups.

lipid levels that were indistinguishable from those of a control group matched for body fat. This may relate to matching of the central abdominal fat depot, a recognized determinant of cardiac risk (20). Despite average initial lipid levels in both groups, modest caloric restriction and fat loss resulted in a less atherogenic profile, highlighting the role diet may play in reducing cardiac risk in such subjects (21).

That the lipid profile improves with weight loss is established (22,23), but no clear evidence exists on the relative effects of caloric restriction and weight loss or whether the outcome is influenced by the presence of diabetes. While we endeavored to separate energy restriction from weight loss, there was weight loss after 4 days of the diet (1.8 ± 0.3 kg), but this is likely to rep-

resent mainly fluid losses secondary to glycogen depletion and some protein loss (24,25). With energy restriction, we found significant improvements in total TGs, VLDL cholesterol, and LDL particle size (Fig. 1). Once significant fat loss, consistent with prediction from energy intake (26), occurred, there were reductions in total and LDL cholesterol, LDL apoB, and free and esterified LDL cholesterol levels (Fig. 2).

Previous studies are consistent with our findings: after 7–10 days of caloric restriction (8,27) or a 3-day protein sparing fast (28), TGs were lowered by energy restriction. Recently, insulin resistance has been shown to be associated with failure of the liver to suppress release of TG-rich VLDL (29), and it was postulated that insulin normally acts by suppressing TG-rich VLDL



**Figure 3**—Relationship between abdominal fat loss and reduction in (A) LDL free cholesterol (r = 0.65, P = 0.006) and (B) LDL apoB (r = 0.64, P = 0.008) after 4 weeks of hypocaloric diet. NGT; ○, type 2 diabetes; ●.

synthesis. Consistent with this, there was a significant relationship between the improvement in hepatic insulin action (reported previously in Markovic et al. [9]) and the fall in TG levels at d4 ( $\Delta$  hepatic glucose output vs.  $\Delta$  TG: r = 0.55, P = 0.03).

It is noteworthy that the early improvement in the atherogenic profile was not related to the change in energy intake (absolute or relative), despite the large range of energy intake changes, implying that more commonly achieved, less severe reductions in energy intake should produce similar benefits.

Fat loss appears to affect lipids by different mechanisms. The loss of abdominal fat alone, independent of changes in total fat, was strongly associated with falls in LDL apoB and LDL free cholesterol (Fig. 3). In the small number of relevant studies, there are suggestions that abdominal fat may influence lipid changes with fat loss (30,31). Using computed tomography, changes in visceral fat with weight loss were related to lipid and metabolic improvements independent of changes in total fat (30). However, in the latter study

(31), once changes in total fat were taken into account, there was no relationship between abdominal fat loss (measured by magnetic resonance imaging) and lipid improvements, apart from HDL changes and visceral fat loss in women. Other negative studies (23,32) used less accurate estimates of central fat, such as WHR (33). The relationship we found between reduction in apoB and fall in abdominal fat highlights the importance of this fat depot with respect to cardiac risk (34).

No significant changes in HDL cholesterol were found. HDL has been reported to increase (7), decrease (23,30), or remain stable (6) with weight loss. The discrepancy in results seems to be due to the divergent effects of weight loss, which increases HDL, and reduced fat intake, which decreases HDL (35). Because subfractions were not measured in the present study or in previous studies, differential effects on HDL 2 and 3 may have been missed.

Enhanced oxidizability of LDL is associated with type 2 diabetes (2) and may result in unregulated LDL uptake by scavenger receptors, leading to plaque formation. However, there were no significant changes in LDL oxidizability during the study. LDL oxidizability (lag time) in this study is shorter than that reported by some groups; however, because the method is standardized within each laboratory, lag times vary and absolute values are not comparable. While some laboratories report lag times of many hours, other groups report much shorter lag times of around 1 h (36,37), similar to our laboratory. The absence of changes in LDL oxidizability in this study probably relates to the normal initial findings, although LDL oxidation may relate more to the antioxidant content of LDL or the amount of LDL oxidized in vivo (38).

In conclusion, in obese subjects with mild metabolic disturbance, both energy restriction and fat loss improve the lipid profile. Energy restriction, regardless of degree, was associated with lowering of TG-related factors in association with an improvement in hepatic insulin action. Loss of fat from the central abdominal depot was alone associated with improvement in LDL free cholesterol and LDL apoB levels independent of total fat loss. This study clarifies the effects of energy restriction and fat loss and demonstrates how dietary intervention can improve the lipid profile in obese subjects at risk of atherosclerotic vascular disease.

**Acknowledgments** — This study was supported by a block grant and scholarship (to T.P.M.) from the National Health and Medical Research Council of Australia.

We gratefully acknowledge the assistance of M. Kinloch, L. Crampton, and J. Ruys for clinical and laboratory support. We thank Nutri-Metics and Vitaglow for supplying the formula diet and vitamin supplements, respectively.

## References

- Laakso M: Epidemiology of diabetic dyslipidemia. *Diabetes Rev*3:408–422, 1995
- Bierman EL: Atherogenesis in diabetes. *Arterioscler Thromb*12:647–656, 1992
- Bowie A, Owens D, Collins P, Johnson A, Tomkin GH: Glycosylated low density lipoprotein is more sensitive to oxidation: implications for the diabetic patient. *Atherosclerosis*102:63–67, 1993
- Kissebah AH, Vydellingum N, Murray R, Evans DJ, Hartz AJ, Kalkhoff RK, Adams PW: Relation of body fat distribution to metabolic complications of obesity. *J Clin Endocrinol Metab*54:254–260, 1982
- Wing RR, Koeske R, Epstein LH, Nowalk MP, Gooding W, Becker D: Long-term effects of modest weight loss in type II diabetic patients. *Arch Intern Med*147:1749–1753, 1987
- Sonnichsen AC, Richter WO, Schwandt P: Benefit from hypocaloric diet in obese men depends on the extent of weight-loss regarding cholesterol, and on a simultaneous change in body fat distribution regarding insulin sensitivity and glucose tolerance. *Metabolism*41:1035–1039, 1992
- Hughes TA, Gwynne JT, Switzer BR, Herbst C, White G: Effects of caloric restriction and weight loss on glycemic control, insulin release and resistance, and atherosclerotic risk in obese patients with type II diabetes mellitus. *JAMA*77:7–17, 1984
- Kelley DE, Wing R, Buonocore C, Sturis J, Polonsky K, Fitzsimmons M: Relative effects of calorie restriction and weight loss in non-insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab*77:1287–1293, 1993
- Markovic TP, Jenkins AB, Campbell LV, Furler SM, Kraegen EW, Chisholm DJ: The determinants of glycemic responses to diet restriction and weight loss in obesity and NIDDM. *Diabetes Care*21:687–694, 1998
- The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care*20:1183–1197, 1997
- Jenkins AB, Markovic TP, Fleury A, Campbell LV: Carbohydrate intake and short-term regulation of leptin in humans. *Diabetologia*40:348–351, 1997
- Durnin J, Womersley J: Body fat assessed from total body density and its estimation from skinfold thickness: measurements of 481 men and women aged from 16 to 72 years. *Br J Nutr*32:77–79, 1974
- Simons LA, von Konigsmark M, Balasubramaniam S: What dose of vitamin E is required to reduce susceptibility of LDL to oxidation? *Aust NZ J Med*26:496–503, 1996
- Havel RJ, Eder HA, Bragdon JH: The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest*34:1345–1353, 1955
- Bachorik PS, Cloey TA: Rate immunonephelometry and radial immunodiffusion compared for apolipoproteins AI and B assay. *Clin Chem*35:2236–2241, 1989
- Brown MS, Dana SE, Goldstein JL: Cholesterol ester formation in cultured human fibroblasts: stimulation by oxygenated sterols. *J Biol Chem*250:4025–4027, 1975
- Krauss RM, Burke DJ: Identification of multiple subclasses of plasma LDL in normal individuals. *J Lipid Res*23:97–104, 1982
- Stewart MW, Laker ME, Dyer RG, Game F, Mitcheson J, Winocour PH, Alberti KGMM: Lipoprotein compositional abnormalities and insulin resistance in type II diabetic patients with mild hyperlipidemia. *Arterioscler Thromb*13:1046–1052, 1993
- Barakat HA, Carpenter JW, McLendon VD, Khazanie P, Leggett N, Heath J, Marks R: Influence of obesity, impaired glucose tolerance, and NIDDM on LDL structure and composition: possible link between hyperinsulinemia and atherosclerosis. *Diabetes*39:1527–1533, 1990
- Despres J-P, Moorjani S, Lupien PJ, Tremblay A, Nadeau A, Bouchard C: Review: regional body fat, plasma lipoproteins and cardiovascular disease. *Arteriosclerosis*10:497–511, 1990
- Sacks FM, Pfeffer MA, Moye LA, Rouleau JL, Rutherford JD, Cole TG, Brown L, Warnica JW, Arnold JMO, Wun C, Davis BR, Braunwald E: The effect of pravastatin on coronary events after myocardial infarction in patients with average cholesterol levels. *N Engl J Med*335:1001–1009, 1996
- Olefsky J, Reaven GM, Farquar JW: Effects of weight reduction on obesity: studies of lipid and carbohydrate metabolism in normal and hyperlipoproteinemic subjects. *J Clin Invest*53:64–76, 1974
- Wing RR, Jeffery RW, Burton LR, Thorson C, Kuller LH, Folsom AR: Change in waist-hip ratio with weight loss and its association with change in cardiovascular risk factors. *Am J Clin Nutr*55:1086–1092, 1992
- Bray GA, Gray DS: Treatment of obesity: an overview. *Diabetes Metab Rev*4:653–679, 1988
- Burgess NS: Effect of a very-low-calorie diet on body composition and resting metabolic rate in obese men and women. *J Am Diet Assoc*91:430–434, 1991
- Stanko RT, Tieze DL, Arch JE: Body com-

- position, nitrogen metabolism and energy utilization with feeding of mildly restricted (4.2MJ/d) and severely restricted (2.1MJ/d) isonitrogenous diets. *Am J Clin Nutr* 56:636-640, 1992
27. Weinsier RL, James LD, Darnell BE, Wooldridge NH, Birch R, Hunter GR, Bartolucci AA: Lipid and insulin concentrations in obese postmenopausal women: separate effects of energy restriction and weight loss. *Am J Clin Nutr* 56:44-49, 1992
28. Felber J-P, Meyer HU, Curchod B, Maeder E, Pahud P, Jequier E: Effect of a 3-day fast on glucose storage and oxidation in obese hyperinsulinemic diabetics. *Metabolism* 30:184-189, 1981
29. Malmstrom R, Packard CJ, Caslake M, Bedford D, Stewart P, Yki-Jarvinen H, Shepherd J, Taskinen M-R: Defective regulation of triglyceride metabolism by insulin in the liver in NIDDM. *Diabetologia* 40:454-462, 1997
30. Fujioka S, Matsuzawa Y, Tokunaga K, Kawamoto T, Kobatake T, Keno Y, Kotani K, Yoshida S, Tarui S: Improvement of glucose and lipid metabolism associated with selective reduction of intra-abdominal fat in premenopausal women with visceral fat obesity. *Int J Obes* 5:853-859, 1991
31. Leenen R, Van der Kooy K, Drop A, Seidell JC, Deurenberg P, Weststrate JA, Hautvast JGAJ: Visceral fat loss measured by magnetic resonance imaging in relation to changes in serum lipid levels of obese men and women. *Arterioscler Thromb* 13:487-494, 1993
32. Vansant G, Den Besten C, Weststrate J, Deurenberg P: Body fat distribution and the prognosis for weight reduction: preliminary observations. *Int J Obes* 2:133-140, 1988
33. van der Kooy K, Seidell JC: Review: techniques for the measurement of visceral fat: a practical guide. *Int J Obes* 17:187-196, 1993
34. Wald NJ, Law M, Watt HC, Wu T, Bailey A, Johnson AM, Craig W, Ledue TB, Haddow JE: Apolipoproteins and ischaemic heart disease: implications for screening. *Lancet* 343:75-79, 1994
35. Leenen R, Van der Kooy K, Meyboom S, Seidell JC, Deurenberg P, Weststrate JA: Relative effects of weight loss and dietary fat modification on serum lipid levels in the dietary treatment of obesity. *J Lipid Res* 34:2183-2191, 1993
36. Abbey M, Nestel PJ, Baghurst PA: Antioxidant vitamin and low-density-lipoprotein oxidation. *Am J Clin Nutr* 58:525-532, 1993
37. Princen HMG, van Duyvenvoorde W, Buytenhek R, van der Laarse A, van Poppel G, Gevers Leuven JA, van Hinsbergh VWM: Supplementation with low doses of vitamin E protects LDL from lipid peroxidation in men and women. *Arterioscler Thromb Vasc Biol* 5:325-333, 1995
38. Bellomo G, Maggi E, Poli M, Agosta FG, Bollati P, Finardi G: Autoantibodies against oxidatively modified low-density lipoproteins in NIDDM. *Diabetes* 44:60-66, 1995