Metabolism
Clinical and Experimental

VOL 53, NO 9 SEPTEMBER 2004

Effects of Weight Loss in Obese Subjects With Normal Fasting Plasma Glucose or Impaired Glucose Tolerance on Insulin Release and Insulin Resistance According to a Minimal Model Analysis

Yuriko Yoshida, Naotake Hashimoto, Yoshiharu Tokuyama, Hiroshi Kitagawa, Kazuo Takahashi, Kazuo Yagui, Azuma Kanatsuka, Hideaki Bujo, Mayumi Higurashi, Saori Miyazawa, Shouji Yoshida, and Yasushi Saito

We investigated effects of weight loss from diet and exercise regimen in obese subjects with normal fasting plasma glucose or impaired glucose tolerance (IGT) on insulin release capacity and insulin sensitivity. Eight subjects were recruited among visceral obesity patients (4 men, 4 women; age range, 24 to 57 years; body mass index [BMI], 32.8 to 60.3 kg/m^2). All were admitted to Chiba University Hospital for 2 weeks, were treated with a tapering 5,023 to 2,930 kJ diet, and were given exercise equivalent to 628 kJ/d. For assessments, we used a combination of C-peptide secretion rate determination and minimal model analysis as previously reported. BMI and visceral fat area (V) significantly decreased (BMI on initiation *v* after intervention, 43.0 ± 3.2 *v* 40.3 ± 3.1 kg/m^2, $P < .05$; V, 224 ± 22 *v* 188 ± 22 cm^2; $P < .05$). Fasting immunoreactive insulin (F-IRI) and leptin concentrations decreased significantly. Capacity for insulin release in response to glucose increased in all subjects (first-phase insulin secretion [CS1], 4.66 ± 4.05 *v* 6.81 ± 4.57 ng/mL/5 min, $P < .05$), but the insulin sensitivity index (S_i) did not change significantly. These data suggest that weight reduction early in development of type 2 diabetes can oppose progression of diabetes by improving capacity for insulin release.

TYPE 2 DIABETES mellitus is a common metabolic disorder characterized by insulin resistance and deterioration of β-cell function.[1] Early in disease development, visceral fat accumulation is believed to contribute to insulin resistance, leading to clinical onset.[2-5] Weight loss has been found effective in preventing onset of diabetes,[6,7] but whether this occurs via increased insulin sensitivity or changes in insulin release has not been clarified. Several reports have examined β-cell function and insulin action in this context.[8-14] We previously reported that type 2 diabetic patients and non-obese subjects with impaired glucose tolerance (IGT) showed a significant decrease in the first-phase of insulin secretion from the pancreas in intravenous glucose tolerance tests (IVGTT)[15] using a 2-compartment model analysis of C-peptide kinetics representing a modification previously reported.[8-10]

For measurement of insulin sensitivity, glucose clamp studies have been considered the best available method or gold standard.[11] Minimal model analysis has been proposed by Bergman et al[12] as a more practical alternative, while Finegood et al[13,14] developed an insulin-modified minimal model protocol for subjects with diminished insulin secretory function. We have described distinctive pathophysiologic phenotypes among Japanese subjects using a combination of C-peptide secretion rate and minimal model analyses.[16]

A recent examination of the relationship between visceral fat accumulation, insulin secretion, and insulin sensitivity showed that in obese subjects insulin sensitivity was impaired and β cells responded in an exaggerated manner to glucose.[17] The early stages of development of type 2 diabetes are believed to be characterized by decreased insulin sensitivity together with impaired insulin secretion, but changes in insulin pathophysiology in response to a decrease in visceral adipose tissue induced by diet have not been fully elucidated. In the present study of obese subjects with IGT or type 2 diabetes, but with normal or impaired fasting plasma glucose, we examined the

From the Departments of Clinical Cell Biology and Applied Translational Research, Graduate School Medicine, Chiba University, Chiba; Departments of Diabetes and Metabolic Disease and Immunology and Internal Medicine, Asahi General Hospital, Chiba; and the Diabetes Center, Kasori Hospital, Chiba, Japan.

Submitted March 11, 2003; accepted April 16, 2004.

Address reprint requests to Naotake Hashimoto, MD, PhD, Department of Diabetes and Metabolic Disease, Asahi General Hospital, I-1326, Asahi, Chiba, 289-2511, Japan.

0026-0495/04/5309-0014$30.00/0
doi:10.1016/j.metabol.2004.04.002

Table 1. Patient Profiles

Case	Age (yr)	BW (kg) (BMI) B	BW (kg) (BMI) A	V/S B	V/S A	V (cm^2) B	V (cm^2) A	S (cm^2) B	S (cm^2) A	FPG (mg/dL) B	FPG (mg/dL) A	F-IRI (μU/mL) B	F-IRI (μU/mL) A	F-CPR (ng/mL) B	F-CPR (ng/mL) A	HT	HL
1	36	101.7 (38.2)	94.0 (35.3)	0.68	0.57	262	222	386	393	125	93	17.0	12.0	3.1	3.3	−	−
2	57	99.5 (38.8)	94.0 (36.7)	0.58	0.37	283	192	488	509	125	113	9.8	6.8	3.1	2.4	+	−
3	45	97.2 (32.8)	92.4 (31.2)	1.01	1.12	242	256	238	228	115	102	7.0	7.6	3.1	2.1	−	+
4	26	147.4 (49.8)	138.0 (46.6)	0.45	0.39	311	273	691	701	98	78	16.0	10.0	4.6	4.6	−	−
5	27	126.8 (39.3)	118.3 (36.7)	0.37	0.31	136	130	363	417	95	89	18.0	9.9	4.8	3.1	−	−
6	27	147.0 (48.5)	137.7 (45.5)	0.23	0.25	200	97	858	396	82	95	30.1	32.4	3.8	5.9	−	−
7	24	158.2 (60.3)	150.5 (57.0)	0.23	0.19	155	151	911	794	95	85	16.9	15.0	3.9	3.1	−	−
8	46	84.0 (35.6)	78.0 (33.1)	0.58	0.73	201	179	348	246	99	85	14.8	10.3	3.2	3.9	−	−

Abbreviations: B, before intervention; A, after intervention; BW, body weight; V/S, visceral fat subcutaneous fat ratio; V, visceral fat area; S, subcutaneous fat area; FPG, fasting plasma glucose, F-IRI, fasting immunoreactive insulin; F-CPR, fasting C-peptide; HT, hypertension; HL, hyperlipidemia.

effects of therapeutically induced weight loss on first-phase insulin secretion (CSI) and insulin sensitivity using a combination of C-peptide secretion rate measurements and minimal model analysis.

SUBJECTS AND METHODS

Eight subjects with visceral obesity participated in this study (4 men, 4 women; age range, 24 to 57 years; body mass index) [BMI], 32.8 to 60.3 kg/m^2). Five subjects had normal fasting plasma glucose concentrations, while 3 subjects had impaired fasting concentrations (Table 1). One subject had hypertension and was undergoing treatment with benidipine and betaxolol, while another subject who had hyperlipidemia was treated with bezafibrate and pravastatin; other subjects had received no medication related to metabolic disorders (Table 1). A 75-g oral glucose tolerance test (OGTT) showed a diabetic pattern in 4 subjects, and IGT in 4 (Table 2). All subjects were admitted for 2 weeks to Chiba University Hospital, where they were treated with a tapering 5,023 to 2,930 kJ diet to decrease body weight at an essentially steady rate. All subjects were provided 58 to 64 g protein/day, and a nonprotein energy content of 50% to 58% carbohydrate and 20% to 27% fat. All subjects underwent exercise every day, representing an estimated energy expenditure of 628 kJ/d (Fig 1). On study days 3 and 14, we examined insulin sensitivity index (S_i) and CS1 using minimal model analysis as reported previously.[16] Briefly, after 12 hours of overnight fasting, intravenous cannulas were placed in both antecubital veins and kept open with 0.9% saline infusion. A bolus of 50% glucose (25 g) was injected over 1 minute at time 0. Regular human insulin (0.06 U/kg) dissolved in 10 mL 0.9% saline was injected for 30 seconds at 20 minutes. Blood samples were collected at −5, 0, 2, 3, 5, 7, 10, 15, 20, 22, 23, 25, 27, 30, 40, 50, 60, 70, 80, 90, and 100 minutes for determination of plasma glucose and insulin concentrations. C-peptide concentrations were measured at −5, 0, 2, 3, 5, 7, 10, 15, and 20 minutes C-peptide secretion rate (CSR) was estimated mathematically from C-peptide concentrations by deconvolution with a 2-compartment model for C-peptide disappearance kinetics. The S_i parameter was calculated using glucose and insulin concentrations during the IVGTT by a minimal model software program that we developed according to an algorithm described previously by Pacini and Bergman.[18] On study days 2 and 14, we performed blood sampling for measurement of leptin, tumor necrosis factor (TNF)-α, plasminogen activator inhibitor (PAI)-1, and immunoreactive insulin (IRI). Diabetes and IGT were diagnosed by World Health Organization (WHO) criteria. Data are expressed as the mean ± SEM. Plasma leptin concentrations were measured by a human leptin radioimmunoassay kit (LINCO Research, St. Charles, MO) and TNF-α was measured by a human TNF-α immunoassay kit (R&D Systems, Minneapolis, MN). PAI-1 and C-peptide, respectively, were determined by an LPIA-tPAI assay (Diatron, Tokyo, Japan) and a C-peptide enzyme immunoassay (EIA) kit (Eiken, Tokyo, Japan). Sets of data were compared using nonparametric tests. This study was conducted according to the principles expressed in the Declaration of Helsinki.

Table 2. 75-g OGTT

Case	Sex		Before	30 Minutes	60 Minutes	120 Minutes
1	F	PG (mg/dL)	99	164	242	261
		IRI (μU/mL)	15.6	37.2	51.0	80.7
2	F	PG (mg/dL)	105	182	235	221
		IRI (μU/mL)	5.5	23	34	46
3	M	PG (mg/dL)	100	206	236	140
		IRI (μU/mL)	8.0	64.4	130.6	119.4
4	M	PG (mg/dL)	106	204	237	172
		IRI (μU/mL)	18	55	109	98
5	M	PG (mg/dL)	100	133	137	202
		IRI (μU/mL)	13.4	48.5	47.4	212.0
6	M	PG (mg/dL)	86	132	152	146
		IRI (μU/mL)	30.5	156.6	185.8	204.2
7	F	PG (mg/dL)	85	148	193	188
		IRI (μU/mL)	20.9	64.2	65.3	107.3
8	F	PG (mg/dL)	95	168	198	209
		IRI (μU/mL)	10.6	75.6	91.0	141.2

Abbreviations: PG, plasma glucose; IRI, immunoreactive insulin; M, male; F, female.

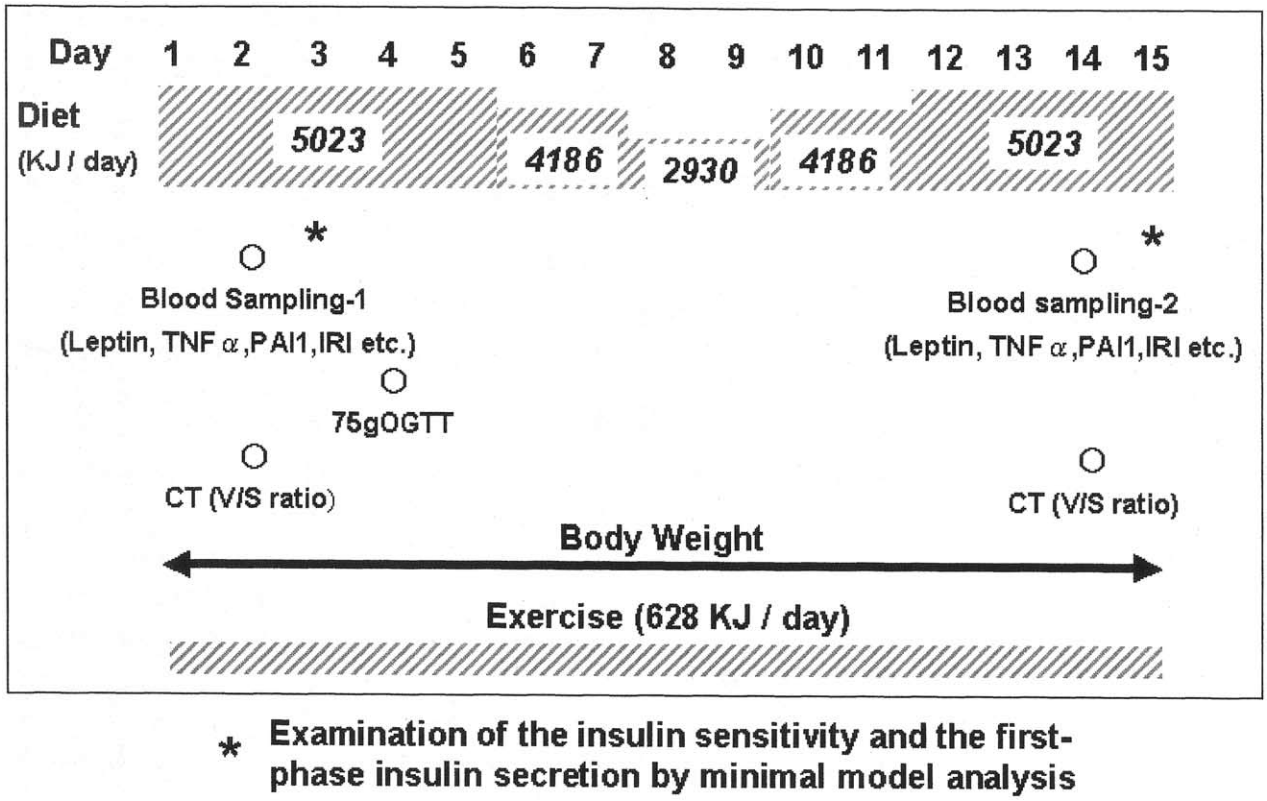

Fig 1. Intervention protocol for reduction of body weight. TNF, tumor necrosis factor; PAI-1, plasminogen actibvator inhibitor-1; CT, computed tomography; V/S, visceral fat area/subcutaneous fat area. *Examination of the insulin sensitivity and the first-phase insulin secretion by minimal model analysis.

RESULTS

Changes in Body Weight, BMI, and Visceral Fat Area

After intervention with diet and exercise for 2 weeks, BMI was decreased (43.0 ± 3.2 at initiation of study *v* 40.3 ± 3.1 on day 14 $P < .05$; Tables 2 and 3). Subjects had lost an average of 7.3 kg at the end of treatment, and visceral fat area measured by computed tomography (CT) decreased from 224.1 ± 21.8 to 187.8 ± 21.6 cm^2 ($P < .05$). Subcutaneous fat area did not change significantly (536.3 ± 89.2 *v* 460.8 ± 71.9 cm^2).

Characteristics of Metabolic Changes From Baseline Following Weight Reduction

After reduction of weight, the 2-hour postprandial PAI-1 concentration significantly decreased ($P < .05$), while fasting PAI-1 did not change significantly. Fasting IRI decreased significantly (16.2 ± 2.4 *v* 13.0 ± 2.9 μU/mL; $P < .05$). The fasting leptin concentration ($P < .05$) and 2-hour postprandial leptin concentration ($P < .05$) decreased significantly.

S_i and Insulin Secretion Rate

Figure 2 shows changes in S_i and CS1 in subjects. After reduction of weight, changes in S_i varied among subjects (1.18 ± 0.32 at initiation *v* 0.95 ± 0.24 × 10^4/min/[μU/min] on day 14; not significant [NS]), but an increase in CS1 was observed in all subjects (4.97 ± 1.6 *v* 6.74 ± 1.86 ng/mL/5min;

Table 3. Metabolic Changes Associated With Intervention Concerning Body Weight

	Basal	After	*r*	*P*
Body weight (kg)	120.2 ± 10.0	112.9 ± 9.5	−2.524	.0116*
BMI	43.0 ± 3.2	40.3 ± 3.1	−2.521	.0117*
V/S	0.518 ± 0.092	0.490 ± 0.11	−0.700	.4838*
V (cm^2)	224.1 ± 21.8	187.8 ± 21.6	−2.100	.0357*
S (cm^2)	536.3 ± 89.2	460.8 ± 70.9	−0.700	.4838
FPG (mg/dL)	106.4 ± 6.9	92.5 ± 3.9	−2.033	.0421*
2h PG (mg/dL)	129.6 ± 11.9	131.5 ± 8.1	−0.507	.6121
F-CPR (ng/mL)	3.6 ± 0.3	4.4 ± 0.7	−0.105	.9165
2h CPR (ng/mL)	6.8 ± 1.1	9.5 ± 1.0	−1.572	.1159
F-IRI (μU/mL)	16.2 ± 2.4	13.0 ± 2.9	−1.960	.0499*
2h IRI (μU/mL)	35.4 ± 3.6	57.1 ± 10.7	−1.690	.0910
F-TNF-α (pg/mL)	12.2 ± 6.7	9.0 ± 3.8	−0.730	.4652
2h TNF-α (pg/mL)	9.5 ± 5.9	10.8 ± 5.4	−1.095	.2733
F-leptin (ng/mL)	19.1 ± 4.6	18.2 ± 4.6	−2.197	.0280*
2h leptin (ng/mL)	19.2 ± 3.8	15.9 ± 3.8	−2.371	.0178*
F-PAI-1 (ng/mL)	100.5 ± 14.1	70.3 ± 7.3	−1.782	.0747
2h PAI-1 (ng/mL)	70.8 ± 13.0	35.9 ± 6.9	−1.992	.0464*
TG (mg/dL)	184.8 ± 52.8	126.3 ± 25.1	−1.820	.0687
FFA (mEq/L)	0.84 ± 0.05	0.82 ± 0.08	−0.254	.7995
T-cho (mg/dL)	185.8 ± 13.0	174.3 ± 12.3	−1.400	.1614

Abbreviations: F, fasting; 2h, 2-hour postprandial; TG, triglyceride; FFA, free fatty acid; T-cho, total cholesterol.

*$P < .05$.

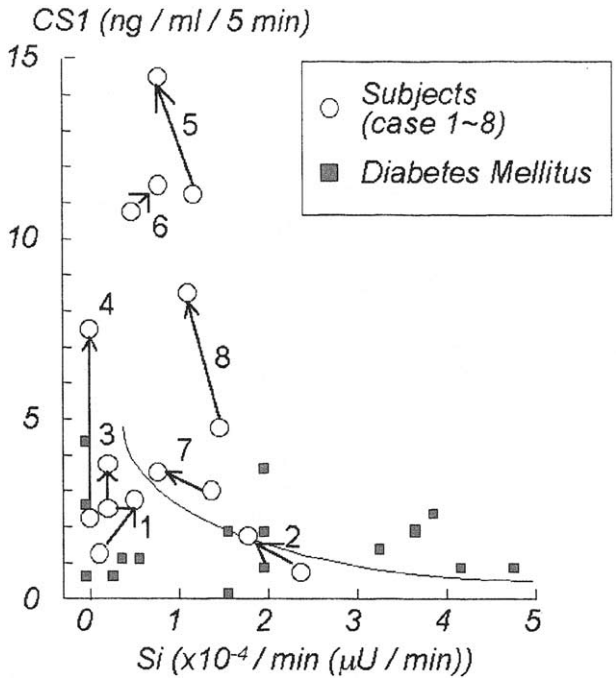

Fig 2. Relationship between S_i and CS1. (O) Indicate obese subjects that we presently studied (cases 1 to 8). CS1 increased in all subjects after weight-loss intervention for 2 weeks. (■) Diabetic subjects as we previously reported.

$P < .05$). Figure 3 shows insulin secretion data from −5 to 20 minutes in an IVGTT of 2 representative subjects. After reduction of weight, C-peptide, after injection of 50% glucose, had increased.

DISCUSSION

The pathogenesis of type 2 diabetes is multifactorial; obesity and more specifically, central body (visceral) fat distribution have been implicated as important contributors. Unfavorable dietary and exercise habits therefore are involved in development of type 2 diabetes.[19] Indeed, such changes in diet have been suspected to underlie the increasing prevalence of type 2 diabetes.[20] Some studies of subjects with IGT have suggested that dietary fat may be particularly important to the development of type 2 diabetes.[21] Recently, a diabetes prevention program (DPP) research group has reported reduction in occurrence of type 2 diabetes by lifestyle intervention or treatment with metformin or acarbose.[6,22] In the DPP report, glycosylated hemoglobin (HbA_{1c}) values paralleled changes in body weight, suggesting major pathogenic importance in type 2 diabetes. However, whether beneficial effects of weight reduction involve insulin sensitivity, insulin release, or both has not been clear. We suspected that reduction of body weight by diet and exercise would increase insulin sensitivity. Indeed, we excluded subjects with fasting hyperglycemia to avoid effects of glucotoxicity on insulin sensitivity and insulin release. Additionally, some patients with fasting hyperglycemia might take metabolically active medication. Yet, increased insulin release in response to glucose was observed in all subjects, while insulin sensitivity increases were inconstant among subjects.

Several reports have noted that loss of body weight increases insulin sensitivity.[23-26] In our study, after reduction of weight, changes in S_i varied among subjects. We suspect that this disagreement may reflect differences in the intervention period; our study examined short-term effects of weight loss, while most other observations were made over 3 months to 8 years. In our results, after weight reduction, free fatty acids (FFA) and TNF-α did not change significantly. Another difference in methods was that we studied only obese patients with visceral fat accumulation.

Defects in insulin release are consistently present in patients with IGT, and these are believed to play a critical role in progression from IGT to diabetes mellitus.[27] In this study, we determined CSR by calculations based on the kinetic equation of a 2-compartment model for C-peptide. Most insulin secretion after intravenous glucose administration occurred within CSI (0 to 5 minutes). Our results suggest that decreases in this

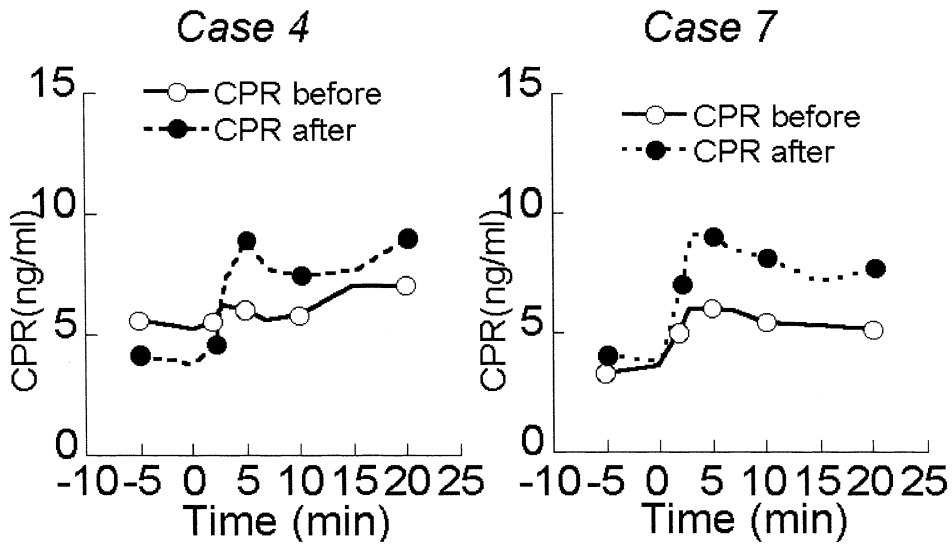

Fig 3. Insulin secretion data from −5 to 20 minutes from the IVGTT (cases 4 and 7).

phase of secretion may be reversible in obese patients with central obesity and IGT or diabetes mellitus with normal fasting glucose concentrations.

Hypertrophy of pancreatic islets and increased insulin secretion have been reported in obesity,[28,29] while several variables have been reported to change with weight reduction. To elucidate factors affecting insulin secretion, we also examined changes in factors having a relationship to insulin-resistant states. FFA were reported to stimulate insulin secretion upon short-term infusion, but inhibit secretion during long-term exposure.[30-32] FFA concentrations did not change significantly in our study. Reported effects of leptin on insulin secretion have varied, with some investigators finding impairment,[33-37] but others noting either enhancement of secretion or pancreatic β-cell proliferation.[38,39] In our study, factors that changed significantly after body weight reduction were the fasting and 2-hour postprandial leptin concentrations. While the specific change underlying our subjects enhanced insulin secretion is difficult to isolate, this might be reduction in circulating leptin reflecting decreased fat mass. Leptin has been reported to inhibit insulin secretion in response to glucose in vivo and in vitro.[40-42] Another possibility is that the weight-loss intervention improved lipid metabolism, which in turn, improved insulin secretion upon stimulation by glucose. Triglyceride (TG) accumulation has been observed in the insulin-resistant state in pancreatic β-cells,[43] while thiazolidine derivatives can prevent lipotoxicity and lipoapoptosis;[44] in the same report, TG content was found to be increased in pancreatic β cells in prediabetic Zucker-diabetic fatty rats. In our study subjects, TG content in pancreatic islets might have decreased as a result of intervention and consequent metabolic improvement, allowing recovery of insulin secretion capacity.

In our study, a decrease in 2-hour postprandial PAI-1 was observed. PAI-1, the primary physiologic inhibitor of plasminogen activation in blood, contributes to thrombus formation and progression of atherosclerosis. Elevated plasma PAI-1 concentrations have been reported in association with obesity and insulin resistance, and PAI-1 expression was found to be increased in adipose tissue in animal models.[45,46] Production of PAI-1 by human adipose tissue has been demonstrated, indicating a possible link between visceral fat accumulation and vascular disease.[47] Increases in PAI-1 were reported to abate after weight loss,[48] and our findings were similar. Thus such interventions may have beneficial effects against progression of not only diabetes, but also atherosclerosis.

In conclusion, our study showed that short-term treatment with diet and exercise partly normalized compromised capacity for glucose-stimulated insulin secretion in obese subjects diagnosed with type 2 diabetes or IGT subjects with normal fasting glucose. Our data suggest that weight reduction could arrest early-stage development of type 2 diabetes.

ACKNOWLEDGMENT

We thank Kimiko Amano and Kikue Tanuma for technical assistance.

REFERENCES

1. DeFronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM: A balanced overview. Diabetes Care 15:318-368, 1992

2. Reaven GM: Role of insulin resistance in human disease. Diabetes 37:1595-1607, 1988

3. Kaplan NM: The deadly quartet: Upperbody obesity, glucose intolerance, hypertriglycemia, and hypertension. Arch Intern Med 149: 1514-1520, 1989

4. DeFronzo RA, Ferrannini E: A hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14:173-194, 1991

5. Fujioka S, Matsuzawa Y, Tokunaga K, et al: Contribution of intraabdominal visceral fat accumulation to the impairment of glucose and lipid metabolism in human obesity. Metabolism 36:54-59, 1987

6. Diabetes Prevention Program Research Group: Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 346:393-403, 2002

7. Pan XR, Li GW, Hu YH, et al: Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and diabetes study. Diabetes Care 20:537-544, 1977

8. Faber OK, Hagen C, Binder C, et al: Kinetics of human connecting peptide in normal and diabetic subjects. J Clin Invest 62:197-203, 1978

9. Eaton RP, Allen RC, Schade DS, et al: Prehepatic insulin production in men: Kinetic analysis using peripheral connecting peptide behavior. J Clin Endocrinol Metab 51:520-528, 1980

10. Polonsky KS, Licinio-Paixao J, Given BD, et al: Use of biosynthetic human C-peptide in the measurement of insulin secretion rates in normal volunteers and type 1 diabetic patients. J Clin Invest 77:98-105, 1986

11. Ferrannini E, Mari A: How to measure insulin sensitivity. J Hypertens 16:895-906, 1998

12. Bergman RN, Ider YZ, Bowden CR, et al: Quantitative estimation of insulin sensitivity. Am J Physiol 236:E667-E677, 1979

13. Finegood DT, Hramiak IM, Dupre J: A modified protocol for estimation of insulin sensitivity with the minimal model of glucose kinetics in patients with insulin-dependent diabetes. J Clin Endocrinol Metab 70:1538-1549, 1990

14. Saad MF, Steil GM, Riad-Gabriel M, et al: Method of insulin administration has no effect on insulin sensitivity estimates from the insulin-modified minimal model protocol. Diabetes 46:2044-2048, 1997

15. Kanatsuka A, Makino H, Sakurada M, et al: First phase insulin response to glucose in nonobese or obese subjects with glucose intolerance: Analysis by C-peptide secretion rate. Metabolism 37:878-884, 1988

16. Tokuyama Y, Sakurai K, Yagui K, et al: Pathophysiologic phenotypes of Japanese subjects with varying degrees of glucose tolerance: Using the combination of C-peptide secretion rate and minimal model analysis. Metabolism 50:812-818, 2001

17. Macor C, Ruggeri A, Mazzonetto P, et al: Visceral adipose tissue impaires insulin secretion and insulin sensitivity but not energy expenditure in obesity. Metabolism 46:123-129, 1997

18. Pacini G, Bergman: MINMOD: A computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. Comput Methods Programs Biomed 23: 113-122, 1986

19. Feskens EJM: Nutritional factors and the etiology of non-insulin-dependent diabetes mellitus: An epidemiological overview. World Rev Nutr Diet 69:1-39, 1992

20. Zimmer P: Type 2 (non-insulin-dependent) diabetes—An epidemiological overview. Diabetologia 22:399-411, 1982

21. Marshall JA, Hoag S, Shetterly S, et al: Dietary fat predicts

conversion from impaired glucose tolerance to NIDDM. Diabetes Care 17:50-56, 1994

22. Chiasson JL, Josse RG, Gomis R, et al: Acarbose for prevention of type 2 diabetes mellitus: The STOP-NIDDM randomized trial. Lancet 359:2072-2077, 2002

23. Muscelli E, Camatra S, Catalano C, et al: Metabolic and cardiovascular assssment in moderate obesity: Effect of weight loss. J Clin Endocrinol Metab 82:2937-2943, 1997

24. Sjostrom CD, Peltonen M, Wedel H, et al: Differentated long-term effects of intentional weight loss on diabetes and hypertension. Hypertension 36:20-25, 2000

25. Ross R, Dagnone D, Jones PJH, et al: Reduction in obesity and related comorbid conditions after diet-induced weight loss or exercise-induced weight loss in men. Ann Intern Med 133:92-103, 2000

26. Greco AV, Mingrone G, Giancaterini A, et al: Insulin resistance in morbid obesity. Diabetes 51:144-151, 2002

27. Polonsky KS: Evolution of β-cell dysfunction in impaired glucose tolerance and diabetes. Exp Clin Endocrinol Diabetes 107:S124-127, 1998

28. Hirose H, Maruyama H, Kido K, et al: Alfa- and beta-cell function in obese Zucker (fa/fa) rats: A study with the isolated perfused pancreas. Clin Sci 86:311-316, 1994

29. Milburn JL Jr, Hirose H, Lee YH, et al: Pancreatic β-cells in obesity: Evidence for induction of functional, morphogic and metabolic abnormalities by increased lon-chain fatty acids. J Biol Chem 270: 1295-1299, 1995

30. Crespin SR, Greenough WB III, Steinberg D: Stimulation of insulin secretion by infusion of free fatty acids. J Clin Invest 48:1934-1943, 1969

31. Sako Y, Grill VE: A 48-hour lipid infusion in the rat time-dependently inhibits glucose-induced insulin secretion and B cell oxidation through a process likely couplrd to fatty acid oxidation. Endocrinology 127:1580-1589, 1990

32. Elks ML: Chronic perfusion of rat islets with palmitate suppress glucose-stimulated insulin release. Endocrinology 133:208-214, 1993

33. Widdup G, Bryson JM, Pawlak D, et al: In vivo and in vitro suppression by leptin of glucose-stimulated insulin hypersecretion in high glucose-ded rats. Eur J Endocrinol 143:431-437, 2000

34. Tsiotra PC, Tsigos C, Raptis SA: TNF alfa and leptin inhibit basal and glucose-stimulated insulin secretion and gene transcription in the HIT-T 15 pancreatic cells. Int J Obes Relat Metab Disord 25:1018-1026, 2001

35. Ahren B, Havel PJ: Leptin inhibits insulin secretion induced by cellular cAMP in a pancreatic B cell line (INS-1 cells). Am J Physiol 277:R959-966, 1999

36. Seufert J, Kieffer TJ, Leech CA, et al: Leptin suppression of insulin secretion and gene expression in human pancreatic islets: Implications for the development of adipogenic diabetes mellitus. J Clin Endocrinol Metab 84:670-676, 1999

37. Zhao AZ, Bornfeldt KE, Beavo JA: Leptin inhibits insulin secretion by activation of phosphodiesterase 3B. J Clin Invest 102:869-873, 1998

38. Khan A, Narangoda S, Ahren B, et al: Long-term leptin treatment of ob/ob mice improves glucose-induced insulin secretion. Int J Obes Relat Metab Disord 25:816-821, 2001

39. Islam MS, Sjoholm A, Emilsson V: Fetal pancreatic islets express functional leptin receptors and leptin stimulates proliferation of fetal islet cells. Int J Obes Relat Metab Disord 24:1246-1253, 2000

40. Tsiotra PC, Tsigos C, Raptis SA: TNF alfa and leptin inhibit basal and glucose-stimulated insulin secretion and gene transcription in the HIT-T15 pancreatic cells. Int J Obes Relat Metab Disord 25:1018-1026, 2001

41. Cases JA, Gabriely I, Ma XH, et al: Physiological increase in plasma leptin markedly inhibits insulin secretion in vivo. Diabetes 50:348-352, 2001

42. Widdup G, Bryson JM, Pawlak D, et al: In vivo and in vitro suppression by leptin of glucose-stimulated insulin hypersecretion in high glucose-fed rats. Eur J Endocrinol 143:431-437, 2000

43. Lee Y, Hirose H, Ohneda M, et al: β-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: Impairment in adipocyte-β cell relationships. Proc Natl Acad Sci USA 91:10878-10882, 1994

44. Higa M, Zhou Y-T, Ravazzola M, et al: Troglitazone prevents mitochondrial alterations, β cell destruction, and diabetes in obese prediabetic rats. Proc Natl Acad Sci USA 96:11513-11518, 1999

45. Shimomura I, Funahashi T, Takahashi M, et al: Enhanced expression of PAI-1 in visceral fat: Possible contributor to vascular disease in obesity. Nat Med 2:800-803, 1996

46. Samad F, Loskutoff DJ: Tissue distribution and regulation of plasminogen activator inhibitor-1 in obese mice. Mol Med 2:568-582, 1996

47. Alessi MC, Peiretti F, Morange P, et al: Production of plasminogen activator inhibitor 1 by human adipose tissue: Possible link between visceral fat accumulation and vascular disease. Diabetes 46: 860-867, 1997

48. Mavri A, Stegnar M, Krebs M, et al: Impact of adipose tissue on plasma plasminogen activator inhibitor-1 in dieting obese women. Arterioscler Thromb Vasc Biol 19:1582-1587, 1999