

# The Determinants of Glycemic Responses to Diet Restriction and Weight Loss in Obesity and NIDDM

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**OBJECTIVE** — To examine the mechanisms by which weight loss improves glycemic control in overweight subjects with NIDDM, particularly the relationships between energy restriction, improvement in insulin sensitivity, and regional and overall adipose tissue loss.

**RESEARCH DESIGN AND METHODS** — Hyperinsulinemic glucose clamps were performed in 20 subjects (BMI =  $32.0 \pm 0.5$  [SEM] kg/m<sup>2</sup>, age =  $48.4 \pm 2.7$  years) with normal glucose tolerance (NGT) ( $n = 10$ ) or mild NIDDM ( $n = 10$ ) before and on the 4th (d4) and 28th (d28) days of a reduced-energy ( $1,100 \pm 250$  [SD] kcal/day) formula diet. Body composition changes were assessed by dual energy x-ray absorptiometry and insulin secretory changes were measured by insulin response to intravenous glucose before and after weight loss.

**RESULTS** — In both groups, energy restriction (d4) reduced fasting plasma glucose (FPG) ( $\Delta$ FPG: NGT =  $-0.4 \pm 0.2$  mmol/l and NIDDM =  $-1.1 \pm 0.03$  mmol/l,  $P = 0.002$ ), which was independently related to reduced carbohydrate intake (partial  $r = 0.64$ ,  $P = 0.003$ ). There was a marked d4 increase in percent of insulin suppression of hepatic glucose output (HGO) in both groups ( $\Delta$ HGO suppression: NGT =  $28 \pm 15\%$  and NIDDM =  $32 \pm 8\%$ ,  $P = 0.002$ ). By d28, with  $6.3 \pm 0.4$  kg weight loss, FPG was further reduced (d4 vs. d28) in NIDDM only ( $P = 0.05$ ), and insulin sensitivity increased in both groups ( $P = 0.02$ ). Only loss of abdominal fat related to improvements in FPG ( $r = 0.51$ ,  $P = 0.03$ ) and insulin sensitivity after weight loss ( $r = 0.48$ ,  $P = 0.05$ ). In contrast to insulin action, there were only small changes in insulin secretion.

**CONCLUSIONS** — Both energy restriction and weight loss have beneficial effects on insulin action and glycemic control in obesity and mild NIDDM. The effect of energy restriction is related to changes in individual macronutrients, whereas weight loss effects relate to changes in abdominal fat.

Obesity is present in the majority of people with NIDDM. Dietary restriction leading to weight loss is crucial in NIDDM therapy; however, the mechanisms responsible for the beneficial effects are not fully understood. In particular, the relative importance of energy restriction per se, as opposed to weight loss, is not well defined.

A number of studies have shown marked improvements in glycemia in NIDDM, disproportionate to weight loss and after short periods of energy restriction (1–3). However, the factors responsible for the early benefit of energy restriction on glycemia are not clear, partly because of the difficulty in isolating effects of energy

restriction from those of weight loss when the first measurements are made 7–10 days into the diet (1–3). One study demonstrated clear glycemic benefits of energy restriction (4) with an associated improvement in insulin sensitivity but did not address hepatic versus peripheral contributions or the determinants of basal glycemia.

Similarly, there is difficulty in isolating the effects of weight loss from those of energy restriction (1,2,5,6). In one study in which a separation was attempted, there was evidence of substantial effects on glucose metabolism and insulin sensitivity in obese NIDDM, but it was not clear how these improvements were related to glycemic changes (3). In studies without clear separation, it is likely that much of the benefit is due to weight loss. Even so, the mechanisms linking weight loss to these improvements are not clear. For example, previous studies (3,6,7) examining associations between improvements in glycemia and reduced basal hepatic glucose output (HGO) predate significant refinements in the technique for measuring HGO that have reduced over- and underestimation of HGO under basal and hyperinsulinemic conditions, respectively (8–10).

Evidence supporting a key role of intra-abdominal fat in the pathogenesis of insulin resistance (11–13) has led to suggestions that changes in this depot are important in the beneficial effects of weight loss. A weak independent association between reduction in visceral fat area and improved glucose tolerance in nondiabetic obese women has been demonstrated (14), as has an association with changes in anthropometric measures of central adiposity in obese nondiabetic men (15).

In this study, we attempted to separate the effects of energy restriction from those of weight loss on glycemia, basal glucose flux, and insulin secretion and action in moderately obese subjects with and without NIDDM. Relationships between changes in glycemia and carbohydrate metabolism and changes in substrate levels and oxidation, as well as changes in total and regional body fat, were assessed. The results support the conclusions that early

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**Abbreviations:** AUC, area under the curve; BG, blood glucose; d0, day 0; d4, day 4; d28, day 28; DXA, dual energy X-ray absorptiometer; FFM, fat-free mass; FPG, fasting plasma glucose; HGO, hepatic glucose output; IGST, intravenous glucose stimulation test; *M*, glucose infusion rate; MCR, metabolic clearance rate; NEFA, nonesterified fatty acid; NGT, normal glucose tolerance; NOGD, nonoxidative glucose disposal; OGTT, oral glucose tolerance test; PG, plasma glucose;  $R_a$ , glucose appearance;  $R_d$ , glucose disappearance; WHR, waist-to-hip ratio.

improvements in glycemia relate only to changes in macronutrient intakes, whereas later changes in glycemia and insulin sensitivity relate to changes in abdominal fat.

### RESEARCH DESIGN AND METHODS

#### Subjects

There were 20 obese subjects (BMI =  $31.7 \pm 0.7$  kg/m<sup>2</sup>), 10 with normal glucose tolerance (NGT) and no family history of NIDDM and 10 with mild NIDDM, matched for age, sex (M/F: 10/10), and anthropometry, recruited from the diabetes clinic of St. Vincent's Hospital, Sydney, and through advertisements in the local press. NGT subjects had a fasting plasma glucose (FPG) <5.5 mmol/l (16) or a normal 2-h oral glucose tolerance test (OGTT). Subjects with NIDDM were treated with diet alone ( $n = 7$ ) or with low doses of oral medication (metformin  $n = 1$ , gliclazide  $n = 2$ ); none had clinical evidence of endocrine, cardiac, hepatic, or renal disease. All subjects consumed <20 g alcohol/day and were only accepted if a dietitian considered them well-motivated to lose weight and if they had not dieted for 6 months. There were technical difficulties in a small number of studies such that the data in some cases do not include all 20 subjects. All subjects gave written informed consent, and the study protocol was approved by the Research Ethics Committee of St. Vincent's Hospital.

#### Experimental protocol

The following parameters were assessed at baseline: usual dietary composition and energy expenditure; weight, anthropometry, and body composition (using dual energy x-ray absorptiometry [DXA]); determination of FPG in the NGT group and, if indicated, OGTT; and insulin secretory response with an intravenous glucose stimulation test (IGST). A baseline (day 0 [d0]) hyperinsulinemic euglycemic clamp (17) assessed insulin sensitivity and glucose turnover. A second clamp was performed on day 4 (d4) of the diet to examine effects of energy restriction before substantial weight loss. A third clamp and second DXA scan and anthropometry were undertaken toward the end of the diet period (day 28 [d28]) after weight loss, with a repeat IGST not less than 72 h later. NIDDM subjects monitored blood glucose (BG) (fasting and 2-h postprandial) during the diet, and participants taking oral hypoglycemics discontinued therapy at least 1 week before the study.

#### Diet

Each subject's diet was assessed by the dietitian using 4-day food records, as described previously (18). Subjects were requested not to alter their level of physical activity throughout and were supplied with a formula diet (Nutri-Metics, Sydney, Australia), customized on the basis of body size, age, and energy intake to reduce each person's intake by  $\sim 1,000$  kcal/day (18). This produced a wide variation between individuals in both absolute and proportional changes in macronutrients. Subjects completed daily food records and were weighed and formally reviewed by the dietitian weekly.

#### 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 to derive waist-to-hip ratio (WHR). BMI was calculated as weight (kilograms) divided by height (meters) squared. Total body fat (%) was estimated from skinfold thickness (19).

#### Body composition

DXA (Lunar DPX; Lunar, Madison, WI) was used to measure fat mass, lean tissue (including muscle and fluid), and bone mineral content for the total body and three standard regions: trunk (chest, abdomen, and pelvis), arms, and legs (20). Abdominal fat was estimated: 1) from the lumbar spine DXA measurement using a standard window extending for 4 cm on either side of the L1 to L5 vertebrae, using version 1.3Y DPX-L software (21), giving a fat concentration (%) and 2) from the total body DXA scan using a manually determined window as described previously (13), giving percent and absolute fat mass. This area contains a relatively high intra-abdominal fat content (22) and correlates strongly with insulin sensitivity (13). Results were similar whether abdominal fat changes were analyzed as changes in concentration or absolute mass, so only data on changes in fat concentration are presented.

#### IGST

Studies commenced between 8:00 and 9:00 A.M. after a 12-h fast. A constant infusion of 50% dextrose, at a dose of 0.3 g/kg body wt (maximum 25 g), was administered over 2 min into a large antecubital vein via an 18-

gauge cannula. Arterialized venous blood samples were obtained via a retrograde cannula inserted into a superficial hand vein on the contralateral arm maintained at  $\sim 50^\circ\text{C}$  with a thermostatically controlled heating pad (23). Baseline blood samples were taken 10 min after insertion of the cannulae. The subject then rested for 15 min before commencement of the dextrose infusion ( $t = 0$ ). Samples were taken at  $t = 0, 1, 2, 3, 4, 5, 6, 8,$  and 10 min for plasma glucose (PG) and insulin measurements.

#### Glucose turnover studies

Clamp studies were started at 8:00 A.M., after a 12-h fast. An antecubital venous catheter was inserted for the infusion of 2-<sup>3</sup>H- and 6-<sup>3</sup>H-glucose (Amersham, Buckinghamshire, U.K.), neutral insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) in hemaccel (Polygeline 35 g/l; Hoechst, Behringwerke, Germany), and 10% (wt/vol) dextrose. Dual tracers were used to assess effects of the diet on glucose cycling, but because both groups behaved similarly and there were no significant changes during the study, the data will be reported elsewhere. Arterialized blood sampling was performed as above.

Glucose turnover was assessed with adjusted priming under basal conditions ( $t = 0$ –150 min) (9) and during a hyperinsulinemic (insulin infused at  $0.025 \text{ U} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , producing levels  $\sim 250$  pmol/l) euglycemic clamp ( $t = 150$ –270 min) using labeled glucose infusates (10). NGT subjects were clamped at a BG level of 5 mmol/l, and NIDDM subjects were clamped at the BG level attained after 150 min in the first study and at the same BG level in the subsequent studies.

#### Indirect calorimetry

Indirect calorimetry (Deltatrac; Datex, Helsinki, Finland) was performed from 30 to 60 and from 240 to 270 min to determine glucose and lipid oxidation basally and during hyperinsulinemia (24). Energy expenditure (kilocalories/24 h), substrate oxidation rates (grams/minute) and non-protein respiratory quotient were calculated as previously described (25). Results have been corrected for fat-free mass (FFM) determined by DXA.

#### Analytical measurements

PG was measured by the glucose oxidase method (YSI 23AM Glucose Analyzer; YSI, Yellow Springs, OH). Serum insulin and C-peptide were assayed by radioimmunoassay

**Table 1—Clinical characteristics of subjects**

	NGT subjects	NIDDM subjects
<i>n</i>	10	10
Age (years)	48.4 ± 3.3	48.3 ± 4.4
BMI (kg/m <sup>2</sup> )	31.7 ± 0.7	32.3 ± 0.8
Waist circumference (cm)	98.5 ± 3.5	99.4 ± 2.7
WHR	0.89 ± 0.02	0.87 ± 0.02
Skinfold (% fat)	35 ± 3	36 ± 2
Percent total fat (DXA)	38 ± 3	40 ± 3
Percent abdominal fat (DXA)	43 ± 2	44 ± 1
Fructosamine (μmol/l)	206 ± 4	260 ± 18*

Data are means ± SEM. \*Significantly different from NGT group ( $P < 0.01$ ).

(26). Nonesterified fatty acid (NEFA) levels were measured by enzymatic colorimetry (NEFAC, Wako, Osaka, Japan). Fructosamine was measured as previously described (25). Plasma samples for <sup>3</sup>H-glucose determination were deproteinized with ZnSO<sub>4</sub> and Ba(OH)<sub>2</sub>. 2-<sup>3</sup>H- and 6-<sup>3</sup>H-glucose radioactivity were determined separately by the modified (27) selective enzymatic detritiation method (28).

### Calculations and statistics

Insulin secretory data are presented as areas under the curve (AUCs) calculated as the incremental area from baseline using the trapezoidal method. Glucose turnover rates (glucose appearance [ $R_a$ ] and glucose disappearance [ $R_d$ ]) were calculated using Steele's non-steady state equations (29) separately for 2-<sup>3</sup>H- and 6-<sup>3</sup>H-glucose after smoothing of the plasma <sup>3</sup>H-glucose time course data using the optimal segments (OPSEG) algorithm (30). Glucose turnover data are based on estimates determined using 6-<sup>3</sup>H-glucose. Nonoxidative glucose disposal (NOGD) was calculated as the difference between total glucose disposal (from  $R_d$  of 6-<sup>3</sup>H-glucose) and the rate of glucose oxidation (determined by indirect calorimetry). All measurements of insulin sensitivity and turnover data are corrected for FFM.

Statistical analyses were performed using general purpose software (Statview; Abacus Concepts, Berkeley, CA). Two comparisons, prediet versus d4 and d4 versus d28 (completion), were performed by *t* test. Possible differences between NGT and NIDDM groups were investigated by analysis of variance of variable differences (d4 to prediet, d28 to d4) against group. In most cases, no group effects were found, and data for the two groups (NGT and NIDDM) were combined for analysis.

Where a significant group effect was found, data were analyzed separately. Relationships between continuous variables were assessed using simple and multiple correlation. Unless stated otherwise, results are expressed as means ± SEM.

## RESULTS

### Clinical characteristics

The clinical characteristics of the subjects are presented in Table 1. The groups were matched for age, BMI, waist circumference, WHR, and total and regional body fat.

### Dietary compositional changes

Both groups had a similar dietary composition. There was a significant absolute reduction in intake of all macronutrients, with a ~50% reduction in energy intake (prediet versus during diet: 2,300 ± 170 vs. 1,100 ± 60 kcal,  $P = 0.0001$ ). The proportion of energy from carbohydrates did not alter with dieting (prediet versus 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 versus during diet: 35.7 ± 1.2 vs. 28.8 ± 0.8%), alcohol (6.8 ± 2.1 vs. 1.3 ± 0.8%), and fiber decreased (26 ± 2 vs. 9 ± 1 g). There were changes in the fatty acid profile (prediet versus 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 1.7 ± 2.2 kg weight loss at d4 that largely represents fluid loss secondary to glycogen depletion and some protein loss (31). By the end of the diet, mean weight loss (groups behaved similarly) was 6.3 ±

0.4 kg, as predicted by energy intake (32). There were significant falls in BMI (prediet = 32.0 ± 0.5 kg/m<sup>2</sup> vs. d28 = 29.9 ± 0.5 kg/m<sup>2</sup>,  $P = 0.0001$ ), waist circumference (prediet = 98.9 ± 2.2 cm vs. d28 = 93.5 ± 2.0 cm,  $P = 0.0001$ ), WHR (prediet = 0.88 ± 0.02 vs. d28 = 0.86 ± 0.02,  $P = 0.0007$ ), and percent body fat based on skinfold thickness (prediet = 35.3 ± 1.5 vs. d28 = 33.7 ± 1.6%,  $P = 0.0001$ ). Both groups lost similar amounts of fat from each regional depot and comparable amounts of total fat (2,640 ± 350 g). While mean abdominal fat loss was only 330 ± 60 g, this constituted the largest proportional loss from any depot (decrease of 12.1 ± 2.2 vs. 7.8 ± 2.2% total fat). Anthropometric predictors of weight loss were prediet total weight ( $r = 0.67$ ,  $P = 0.02$ ), baseline abdominal fat ( $r = 0.60$ ,  $P = 0.007$ ), WHR ( $r = 0.65$ ,  $P = 0.003$ ), and waist circumference ( $r = 0.79$ ,  $P = 0.0001$ ). The only anthropometric predictor of abdominal fat loss was WHR ( $r = 0.66$ ,  $P = 0.003$ ).

### Metabolic control and glucose responses

The home BG readings of the NIDDM group (8 subjects) showed a tendency for fasting levels to fall early with a significant fall by d28 (prediet = 7.9 ± 1.2 mmol/l, d4 = 6.5 ± 0.5 mmol/l, and d28 = 5.4 ± 0.2 mmol/l; prediet vs. d4:  $P = 0.10$  and d4 vs. d28:  $P = 0.02$ ). However, postprandial BG fell early with no further reduction (prediet = 9.5 ± 1.1 mmol/l, d4 = 6.5 ± 0.6 mmol/l, and d28 = 5.9 ± 0.2 mmol/l; prediet vs. d4:  $P = 0.004$  and d4 vs. d28:  $P = 0.25$ ).

This improvement in the NIDDM group was confirmed by the significant reductions in formal FPG levels by d4 and between d4 and d28 (Table 2) and fructosamine at d28 (prediet = 260 ± 18 μmol/l vs. d28 = 221 ± 11 μmol/l,  $P = 0.006$ ). In contrast, there was a small significant early fall in FPG in the NGT group, followed by a late increase at d28 (group effect  $P = 0.01$ ). Notably, there was no relationship between the magnitudes of the early (prediet to d4) and the late (d4 to d28) glycemic changes ( $r = 0.11$ ,  $P = 0.65$ ), suggesting that different mechanisms are responsible.

### Insulin secretory changes

As expected, the baseline incremental early phase (AUC  $t = 0$ –10 min) insulin response to intravenous glucose was markedly lower in NIDDM subjects compared with NGT subjects ( $P = 0.03$ ). Weight loss resulted in dichotomous effects: in NGT subjects, the insulin response was significantly reduced

Table 2—Glucose turnover data

	Group	Prediet	d4	d28	Significance of effect*	
					Early	Late
Basal data						
PG (mmol/l)	NGT	5.0 ± 0.1	4.6 ± 0.2	5.1 ± 0.2	0.002	0.07
	NIDDM	7.3 ± 0.7	6.2 ± 0.5	5.3 ± 0.4		0.05
Insulin (pmol/l)	NGT	49 ± 4	29 ± 6	30 ± 5	0.0001	0.43
	NIDDM	82 ± 7	52 ± 8	43 ± 6		
Basal MCR (ml · kg <sup>-1</sup> FFM · min <sup>-1</sup> )	NGT	3.6 ± 0.4	2.7 ± 0.3	3.2 ± 0.2	0.050	0.037
	NIDDM	2.1 ± 0.2	2.0 ± 0.3	2.4 ± 0.2	0.37	
Basal HGO (μmol · kg <sup>-1</sup> FFM · min <sup>-1</sup> )	NGT	17.6 ± 1.9	12.2 ± 1.4	15.8 ± 1.4	0.0002	0.04
	NIDDM	14.0 ± 1.1	11.3 ± 1.3	12.7 ± 1.3		
Clamp data						
PG (mmol/l)	NGT	5.1 ± 0.1	4.9 ± 0.1	4.9 ± 0.1	0.48	0.69
	NIDDM	5.3 ± 0.4	5.4 ± 0.4	5.6 ± 0.4		
Insulin (pmol/l)	NGT	209 ± 14	169 ± 13	175 ± 16	0.0003	0.47
	NIDDM	242 ± 14	194 ± 11	197 ± 11		
Clamp HGO (μmol · kg <sup>-1</sup> FFM · min <sup>-1</sup> )	NGT	7.2 ± 1.1	2.8 ± 1.8	2.2 ± 1.3	0.002	0.87
	NIDDM	3.8 ± 2.1	0.6 ± 1.8	0.7 ± 1.6		
R <sub>d</sub> (μmol · kg <sup>-1</sup> FFM · min <sup>-1</sup> )	NGT	28.3 ± 3	20.9 ± 2.8	23.9 ± 2.8	0.001	0.004
	NIDDM	18.9 ± 2.0	15.8 ± 1.8	19.6 ± 1.9		
NOGD (μmol · kg <sup>-1</sup> FFM · min <sup>-1</sup> )	NGT	13.7 ± 2.4	12.3 ± 2.8	12.1 ± 2.4	0.47	0.49
	NIDDM	5.1 ± 1.7	8.5 ± 1.4	10.6 ± 1.6		
M (μmol · kg <sup>-1</sup> FFM · min <sup>-1</sup> )	NGT	19.8 ± 3.1	18.2 ± 2.8	21.5 ± 3.7	0.76	0.02
	NIDDM	14.4 ± 1.8	15.2 ± 2.3	18.7 ± 2.2		

Data are means ± SEM. For glucose and insulin data: NGT, n = 10; NIDDM, n = 9. For turnover data: NGT, n = 8; NIDDM, n = 9. \*Where there is a significant group effect, each group is analyzed separately; where there is no group effect, data are combined, and groups are analyzed together: early, prediet vs. d4; late, d4 vs. d28.

(prediet = 2,580 ± 546 pmol · l<sup>-1</sup> · min<sup>-1</sup> vs. postdiet = 1,710 ± 246 pmol · l<sup>-1</sup> · min<sup>-1</sup>, P = 0.04, group effect P = 0.02), whereas in NIDDM, there was no significant change (prediet = 996 ± 504 pmol · l<sup>-1</sup> · min<sup>-1</sup> vs. postdiet = 1,068 ± 444 pmol · l<sup>-1</sup> · min<sup>-1</sup>, P = 0.53). These data are consistent with the basal and clamp C-peptide levels. In NGT subjects, fasting C-peptide fell from 1.8 ± 0.1 μg/l to 1.3 ± 0.2 and 1.4 ± 0.3 μg/l at d4 and d28, respectively (P = 0.03 for prediet vs. d4), while in NIDDM, there was no significant change in either period (2.4 ± 0.2, 2.1 ± 0.3, and 1.6 ± 0.2 μg/l for prediet, d4, and d28, respectively, P = 0.06 for d4 vs. d28). Similar changes were seen in clamp C-peptide levels (data not shown).

### NEFA and thermogenic responses

Energy restriction resulted in a significant early increase in basal NEFA and fat oxidation (Table 3). Conversely, basal carbohydrate oxidation was significantly reduced. During hyperinsulinemia, fat oxidation was less suppressed after 3 days, with a return toward prediet levels by d28 and a converse change in carbohydrate oxidation. There was no significant change in insulin-

stimulated carbohydrate oxidation (clamp-basal) over the three studies, nor were there any significant changes in resting or clamp energy expenditure (data not shown). The increase in basal NEFA levels at d4 was related to the reduction in both energy and fat intake (both corrected for FFM) at this time (r = -0.50, P = 0.03 and r = -0.46, P = 0.05, respectively).

### Glucose turnover and insulin sensitivity changes

Prediet basal R<sub>a</sub> was similar in both groups, as was insulin suppression of HGO (Table 2) (NGT versus NIDDM, P = 0.11 and P = 0.19, respectively). However, as expected, total insulin-stimulated R<sub>d</sub> and NOGD were significantly lower in the NIDDM group (NGT versus NIDDM, P = 0.02 and P = 0.009, respectively) (Table 2).

By d4, there was a significant reduction in basal HGO in both groups (Table 2) and a significant enhancement of insulin suppressibility of HGO at d4 (P = 0.002), with no further change between d4 and d28. In both groups, there was an early fall in insulin-stimulated R<sub>d</sub>, which increased toward prediet levels by d28 (Table 2). These changes in R<sub>d</sub> largely reflect the effects

of the diet on glucose oxidation, which also fell at d4 and increased toward baseline levels at d28 (Table 3). Although there is a suggestion of an increase in NOGD in the NIDDM group, neither a group effect nor a significant change in NOGD was detected over the study period (Table 2). Before diet treatment, the glucose infusion rate (M), corrected for FFM, tended to be lower in the NIDDM group (NGT versus NIDDM, P = 0.09). The groups behaved similarly with energy restriction: there was no change in M at d4, but there was a significant increase at d28, largely due to increased glucose oxidation (Table 2).

Caloric restriction significantly reduced fasting insulin in both groups, apparent after only 3 days of dieting (Table 2). There was also a significant early fall in clamp insulin levels in both groups. Thus, the changes in glucose disposal data at lower circulating insulin levels at this time strongly suggest an early improvement in NOGD and an increase in R<sub>d</sub> at d28, particularly in NIDDM. Consistent with this notion, when the NIDDM data (Table 2) were examined in isolation, there was a significant improvement in NOGD at d28 (P = 0.05 vs. prediet), most of which had occurred at d4.

**Table 3—Thermogenic data**

	Group	Prediet	d4	d28	Significance of effect*	
					Early	Late
<b>Basal data</b>						
Carbohydrate oxidation (mg · min <sup>-1</sup> · kg <sup>-1</sup> FFM)	NGT	1.40 ± 0.19	0.32 ± 0.09	0.53 ± 0.18	0.0001	0.12
	NIDDM	1.33 ± 0.22	0.37 ± 0.21	0.74 ± 0.24		
Fat oxidation (mg · min <sup>-1</sup> · kg <sup>-1</sup> FFM)	NGT	1.18 ± 0.12	1.52 ± 0.09	1.46 ± 0.08	0.0001	0.07
	NIDDM	1.17 ± 0.11	1.48 ± 0.08	1.29 ± 0.13		
NEFA (mmol/l)	NGT	0.62 ± 0.09	0.98 ± 0.06	0.84 ± 0.05	0.0002	0.03
	NIDDM	0.63 ± 0.06	0.85 ± 0.05	0.75 ± 0.08		
<b>Clamp data</b>						
Carbohydrate oxidation (mg · min <sup>-1</sup> · kg <sup>-1</sup> FFM)	NGT	2.46 ± 0.27	1.32 ± 0.29	1.94 ± 0.29	0.0001	0.01
	NIDDM	2.49 ± 0.22	1.31 ± 0.20	1.62 ± 0.18		
Fat oxidation (mg · min <sup>-1</sup> · kg <sup>-1</sup> FFM)	NGT	0.68 ± 0.09	1.11 ± 0.08	0.87 ± 0.08	0.0001	0.01
	NIDDM	0.62 ± 0.07	1.03 ± 0.10	0.86 ± 0.10		
NEFA (mmol/l)	NGT	0.15 ± 0.03	0.27 ± 0.04	0.14 ± 0.03	0.0006	0.001
	NIDDM	0.16 ± 0.03	0.24 ± 0.02	0.17 ± 0.03		

Data are means ± SEM. NGT, *n* = 10; NIDDM, *n* = 9. \*Because there are no group effects for this data, *P* values refer to a combined analysis of data from both groups: early, prediet vs. d4; late, d4 vs. d28.

### PG, insulin action, diet, and body composition

When changes in FPG were analyzed in relation to changes in diet, glucose turnover, and body composition, different variables related to the early and late changes in FPG. In simple correlations, the early fall in FPG (prediet to d4) was associated with reduced carbohydrate intake (corrected for each subject's FFM) at this time in both NGT and NIDDM subjects (*r* = 0.49, *P* = 0.03), without any relationship to changes in total energy, fat, or protein intakes (*r* = 0.05 to 0.18, *P* = 0.3 to

0.8). In a partial correlation with the three macronutrients, reduction in carbohydrate was independently associated with the early fall in FPG (partial *r* = 0.64, *P* = 0.003), while reduction in fat was associated with an increase in FPG (partial *r* = -0.54, *P* = 0.02), but again, there was no association with protein (Table 4). Changes in energy and macronutrient intake did not relate to the late changes (d4 to d28) in PG levels. However, at this time, when there were significant changes in body composition (prediet versus postdiet [d28]), loss of abdominal fat

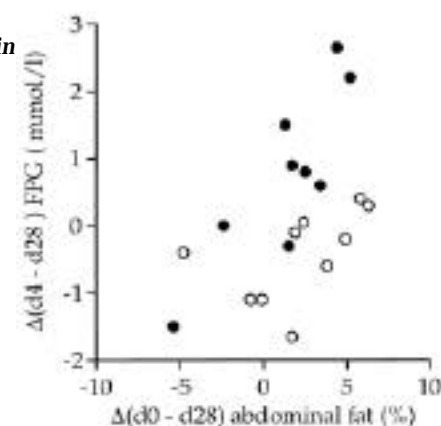
(abdominal window) was the only fat loss that correlated with the late fall in FPG (*r* = 0.51, *P* = 0.025) (Table 4) (Fig. 1).

Since the groups appear to be behaving differently with respect to the late changes in PG (group effect *P* = 0.02; PG increasing in NGT subjects and falling in NIDDM), separate correlations were performed (NIDDM *r* = 0.85, *P* = 0.004 and NGT *r* = 0.50, *P* = 0.14). These correlations suggest that most of the relationship between change in PG and abdominal fat loss is due to the behavior of the NIDDM group (Fig. 1).

**Table 4—Correlation of early and late PG and insulin sensitivity changes with changes in macronutrient intake and regional fat loss**

	Δ FPG		Δ Glucose infusion rate
	Prediet to d4	d4 to d28	d4 to d28
<b>Correlation with Δ macronutrient intake (partial <i>r</i>)</b>			
Protein	0.36	—	—
Fat	-0.54*	—	—
Carbohydrate	0.64†	—	—
<b>Correlation with Δ fat loss (%) from regional depots (<i>r</i>)</b>			
Total	—	0.15	0.23
Arm	—	0.17	0.01
Leg	—	0.15	0.18
Trunk	—	0.18	0.24
<b>Abdominal</b>			
Abdominal window	—	0.51*	0.43
Lumbar spine	—	0.38	0.48*

NGT, *n* = 10; NIDDM, *n* = 9. \**P* < 0.05; †*P* < 0.005.



**Figure 1—Relationship between late change in FPG (d4 to d28) and change in percent abdominal fat content (prediet to d28) (simple regression: *r* = 0.51, *P* = 0.025; when groups are analyzed separately: NIDDM *r* = 0.85, *P* = 0.004 and NGT *r* = 0.50, *P* = 0.14). ○, NGT; ●, NIDDM.**

Abdominal fat loss was also the only fat depot loss that related to the late change in  $M$  ( $r = 0.48$ ,  $P = 0.046$ ): a fall in abdominal fat (lumbar spine window) was associated with an increase in  $M$  across both subject groups (the association with abdominal fat changes measured using the modified abdominal window was close to significance,  $r = 0.43$ ,  $P = 0.06$ ) (Table 4).

**CONCLUSIONS** — Energy restriction resulting in weight loss improves various metabolic parameters in obesity (33,34), particularly when glucose tolerance is abnormal (35,36). There has been controversy regarding the relative effect of energy restriction compared with weight loss, with some studies suggesting a dominant role for reduced energy intake (1–4) and others for weight loss per se (5,37,38). Only one study included subjects with and without NIDDM (37). Our study demonstrates major early changes with energy restriction in obese normal and NIDDM subjects. By d4 of dieting, there was a significant reduction in basal  $R_a$  and a marked increase in insulin suppressibility of HGO in both groups (Table 2). This was accompanied by a significant fall in home-measured postprandial BG readings in NIDDM. An early fall in laboratory-measured FPG occurred on d4, related to concurrent changes in macronutrient intake. By d28, with substantial weight loss, insulin sensitivity increased in all subjects but more markedly in the NIDDM subjects. The additional improvements in PG and insulin sensitivity at d28 correlated with a loss of abdominal fat.

Energy restriction and weight loss thus appear to have independent effects on glycemic control. Reduced carbohydrate intake was strongly related to the early fall (d4) in glycemia in both groups, with reduction of fat intake independently having the reverse association. These associations are consistent with reports in NIDDM of adverse glycemic effects of high carbohydrate diets and beneficial effects of moderate fat diets (39–41). In obesity with and without NIDDM, dietary carbohydrate content influences fasting glycemia (2,42,43). Reduced carbohydrate intake may exert these effects simply by reducing daily glycemic excursion with lower subsequent fasting glucose levels (44). An independent effect of dietary fat on glycemia, which would not be detected in traditional caloric substitution studies, has also been shown. The mechanisms underlying this

are not clear but could include dietary fat slowing carbohydrate absorption (45).

The early (d4), marked improvement in glycemia was associated with changes in basal and insulin-stimulated glucose flux. Basally, there was a decreased HGO, counteracted to a variable extent by decreases in basal metabolic clearance rate (MCR). At d4, insulin suppressibility of HGO was markedly improved, with no further benefit from weight loss in both groups. A possible explanation for the prompt reductions in basal and insulin-suppressed HGO may be the expected fall in hepatic glycogen with reduced carbohydrate intake. Under conditions of negative energy balance, non-insulin-mediated glucose use could exceed the amount of ingested carbohydrate, thereby constricting the glucose pool (46). The early reduction in MCR may be due to competition between glucose and mobilized fatty acids for peripheral metabolism (47). Although changes in MCR did not correlate with changes in lipid related variables, there were clear reciprocal changes in fat and carbohydrate oxidation at this time.

By d28 and with significant weight loss, the groups behaved differently, with FPG decreasing further in the NIDDM subjects and increasing toward prediet levels in control subjects. However, overall, there was improved insulin sensitivity in both, due to a combination of improved hepatic and peripheral insulin sensitivity. As for d4, the late glycemic changes comprised opposing changes in basal HGO and MCR. However, the improvement in glycemia in the NIDDM group at this time relates largely to an increased MCR. Concordant with this interpretation, the major change in insulin sensitivity in this period was  $R_d$ , particularly carbohydrate oxidation. Although  $R_d$  has not apparently improved compared with prediet, the clamp insulin levels were considerably lower during the diet, consistent with improved insulin clearance with energy restriction (48,49). It is therefore reasonable to conclude there has been an enhancement in peripheral insulin action with weight loss. However, no significant improvement in NOGD was demonstrated, perhaps because clamp insulin levels were lower than required for optimal measurement of insulin action on glycogen synthesis. Similar studies with higher clamp insulin levels have shown improvements in NOGD with weight loss (6,7,38) in NIDDM.

A reduction in the abdominal fat depot was associated with both the late increase in

insulin sensitivity and fall in FPG. Notably, only changes in abdominal fat correlated with improved glucose metabolism. Abdominal adipocytes appear to have unique properties, including increased sensitivity to catecholamine-induced lipolysis (50) and higher lipid turnover (51). Subjects with upper-body obesity appear most able to lose fat from this depot (14,52). Confirming this, our strongest predictors of both total and abdominal fat loss were the abdominal fat depot, WHR, and waist circumference. However, this is the first demonstration that improved glucose metabolism with diet-induced weight loss relates specifically to abdominal fat loss. This result adds significance to the well-described association between insulin resistance and central adiposity in cross-sectional studies (11–13).

There was a substantial effect of energy restriction in improving metabolic control in association with a significant increase in lipid oxidation and circulating NEFA and a reduction in oxidative glucose disposal. Diet-induced increases in lipid oxidation are well described with energy restriction (53) and weight loss (54,55). Consistent with the glucose–fatty acid cycle (47), the reduced  $R_d$  was accounted for by reduced carbohydrate oxidation. The improved hepatic insulin action in the face of elevated circulating NEFA levels was unexpected. It is possible that, because of hepatic glycogen depletion secondary to energy restriction, increased gluconeogenesis from elevated NEFAs (56) would have increased glycogen synthesis rather than HGO.

NIDDM subjects had basal and clamp HGO levels similar to those of NGT subjects, consistent with evidence that basal HGO is not elevated in mild NIDDM (9,57,58). Factors that may account for the similar clamp HGO include the mildness of the NIDDM (58), the slightly longer exposure to insulin during the clamps in NIDDM subjects (clamp length NGT versus NIDDM:  $130 \pm 3$  vs.  $143 \pm 5$  min,  $P = 0.03$ ), and their slightly higher clamp PG levels (Table 2). This study was specifically designed to assess hepatic insulin action by the use of relatively low physiologic insulin levels. In most studies where HGO has been measured under hyperinsulinemia (6,7,38), results have not been meaningful because of complete suppression of HGO by hyperinsulinemia ( $>540$  pmol/l). In the only study (5) with a lower insulin level (60 pmol/l), basal and clamp HGO tended to fall with weight loss.

Changes in insulin secretion with the diet were small and unlikely to explain the

metabolic improvements. As previously reported (37,49,59), the NIDDM and NGT groups behaved differently, with the NGT group displaying reduced insulin secretion to intravenous glucose after modest weight loss and the NIDDM group showing no change. As discussed in Polonsky et al. (49), the apparent divergent effect of diet in NGT and NIDDM subjects is due to the relative importance of decreasing insulin sensitivity with related hyperinsulinism (as in NGT) versus that with related insulin deficiency (as in NIDDM).

In conclusion, both energy restriction and weight loss have important beneficial effects on insulin action and glycemic control in obesity and mild NIDDM. The initial effect of energy restriction is related to changes in individual macronutrients, whereas the effect of weight loss appears to relate particularly to changes in abdominal fat depots. The presence of mild NIDDM does not affect the overall response to energy restriction; however, eventual improvement with weight loss is more marked in this group starting with greater metabolic derangement. The importance of carbohydrate reduction in early glycemic improvement suggests that it is responsible for the early improvement in energy restriction in diabetic patients. However, weight loss resulting from caloric restriction produces additional glycemic improvement. Therefore, the immediate benefits of a major lowering of carbohydrate intake on glycemia in NIDDM must be balanced against its probable effect on reducing leptin and thereby increasing appetite (18). This dietary dilemma is not an issue in people with NGT, where the established benefits of a low-fat high carbohydrate (60) diet are not counteracted by adverse glycemic change.

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