Insulin resistance in obese patients with type 2 diabetes mellitus : effects of a very low calorie diet

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CHAPTER 7

Loss of 50% overweight substantially improves insulin sensitivity in obese insulin-treated type 2 diabetic patients using a very low calorie diet.

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ABSTRACT

Calorie restriction *per se* improves hyperglycaemia primarily via a reduction in basal endogenous glucose production (EGP) in obese patients with type 2 diabetes mellitus. To evaluate the effect of weight reduction as opposed to calorie restriction, on insulin sensitivity, 10 obese (body mass index [BMI] 40.2 ± 1.6, mean ± SEM) insulin-treated type 2 diabetic patients (HbA_{1c} 7.7 ± 0.4%, FPG 11.1 ± 0.8 mmol/L) were studied during a very low calorie diet (VLCD, 450 kCal/day) on day 2 and again after losing 50% of their overweight (50% OWR). Oral blood glucose-lowering agents and insulin were discontinued 3 weeks prior to the VLCD and at the start of the VLCD, respectively. EGP and whole-body glucose disposal ([6,6-$^2H_2$]-glucose), lipolysis ([$^2H_5$]-glycerol) and substrate oxidation rates were measured on both study days in basal and hyperinsulinaemic (insulin infusion rate 40mU/m2/min) euglycaemic conditions.

From day 2 to day 50% OWR, weight loss amounted 20.3 ± 2.2 kg. FPG decreased from 12.5 ± 0.5 to 7.8 ± 0.5 mmol/L (p = 0.0001), while basal EGP was restored to normal levels (20.0 ± 0.9 to 16.4 ± 1.2 µmol.kg fat free mass [FFM]$^{-1}$.min^{-1}, p = 0.001). Insulin-stimulated glucose disposal increased from 18.8 ± 2.0 to 39.1 ± 2.8 µmol.kgFFM^{-1}.min^{-1} (p = 0.001), due to an improvement in both oxidative and non-oxidative glucose metabolism. The ability of insulin to suppress EGP also improved: EGP during hyperinsulinaemia decreased from 8.5 ± 0.9 µmol.kgFFM^{-1}.min^{-1} on day 2 to 4.6 ± 1.2 µmol.kgFFM^{-1}.min^{-1} on day 50% OWR. Finally, insulin suppressibility of whole lipolysis also improved as indicated by a lower R_a of glycerol and lower serum glycerol and non-esterified fatty acid concentrations during hyperinsulinaemia on day 50% OWR.

In conclusion, as opposed to caloric restriction *per se*, which only decreases basal EGP, weight loss also considerably improves insulin sensitivity, especially insulin-stimulated glucose uptake, in severely obese insulin-treated type 2 diabetic patients. This occurred despite the fact that all blood glucose-lowering agents were discontinued and patients were still obese (BMI 32.3 kg/m^2). This observation stresses the fundamental importance of dietary intervention in this patient group.

INTRODUCTION

Most type 2 diabetic patients are obese[1]. Insulin resistance plays a pivotal pathogenetic role in inducing and maintaining hyperglycaemia in this patient group and often leads to difficulties in achieving adequate glycaemic regulation.

It is well known that weight reduction improves hyperglycaemia[2-5] in obese patients with type 2 diabetes mellitus. In fact, blood glucose levels decline in response to caloric restriction even before significant weight loss has occurred[2,3,6,7], and improve further with ongoing weight loss[2,8]. In a previous study, we showed that blood glucose levels decline already after 2 days of a very low calorie diet in obese insulin-treated type 2 diabetic patients. The mechanism underlying this blood glucose-lowering effect of a VLCD was a decrease in basal endogenous glucose production (EGP), while hepatic and peripheral insulin sensitivity were unaffected[9].

The present study was conducted to evaluate, again in obese insulin-treated type 2 diabetic patients, whether a prolonged VLCD (Modifast®, 450 kCal/day) leading to substantial weight loss (50% of overweight [50% OWR]) has a different blood glucose-lowering mechanism as compared to caloric restriction only (2-day VLCD). By establishing baseline metabolic status at day 2 of a VLCD, we aimed to largely negate the effects of caloric restriction *per se* so as to specifically determine the impact of body weight reduction. During the study all blood glucose-lowering agents, including insulin, were discontinued. We used [6,6-$^2H_2$]-glucose to measure EGP, and the hyperinsulinaemic euglycaemic clamp technique to assess insulin-mediated peripheral glucose disposal and the capacity of insulin to suppress EGP. In addition, we measured whole-body lipolysis via infusion of [$^2H_5$]-glycerol, and substrate oxidation rates via indirect calorimetry.

RESEARCH DESIGN AND METHODS

Subjects

We studied 10 obese (BMI 40.2 ± 1.6 kg/m^2, mean ± SEM) patients with type 2 diabetes mellitus (FPG 11.1 ± 0.8 mmol/L, HbA_{1c} 7.7 ± 0.4%, duration of type 2 diabetes mellitus 8 ± 3 years), 8 women and 2 men, with a mean age of 54 ± 3 years. Subjects were recruited via local advertisements. All patients underwent a medical screening including a physical examination, resting electrocardiogram and blood chemistry tests to make sure that they were otherwise healthy and did not have liver-or renal function abnormalities. Patients had to use at least 30 units of insulin per day (mean 94 ± 14 units/day; 8 patients also used metformin and 2 patients used rosiglitazone with the insulin therapy) and had to have a BMI > 30 kg/m^2. In addition, patients had to have remaining endogenous insulin secretion defined as a fasting plasma C-peptide level of more than 0.8 ng/mL and/or a 2-fold increase of the basal C-peptide level after administration of 1 mg glucagon i.v.

Patients had to have a stable body weight for at least 3 months and were instructed not to alter life style habits (eating, drinking, exercise) from screening until the start of the study. None of the patients were smokers and the use of other medication (than that used specifically for the treatment of hyperglycaemia) known to alter glucose or lipid metabolism was prohibited.

Written informed consent was obtained from each subject after oral and written explanation of the study had been given. The study was approved of by the Medical Ethical Committee of Leiden University Medical Centre.

Diet and protocol outline

3 weeks prior to the start of the study all oral blood glucose-lowering medication was discontinued. At day -1 only short acting insulin was given, evening doses of intermediate and long acting insulin were omitted. On day 0, patients started a VLCD (450 kCal/day) consisting of 3 sachets of Modifast® (Nutrition & Santé, Antwerpen, Belgium) per day, providing about 50 g protein, 50 to 60 g carbohydrates, 7 to 9 g lipids and 10 g dietary fibres daily.

Insulin therapy remained stopped from the start of the VLCD on. After 48 h of the VLCD, patients were admitted to the research centre for the metabolic studies (day 2) as outlined below. After this study day patients continued the VLCD until they had lost 50% of their overweight (see Calculations*)*. Then the second study day took place (day 50% overweight-reduced [OWR]) (See Fig. 1)

During the VLCD patients visited the research centre on a weekly basis for measurement of body weight, waist-hip ratio, blood pressure and blood glucose regulation.

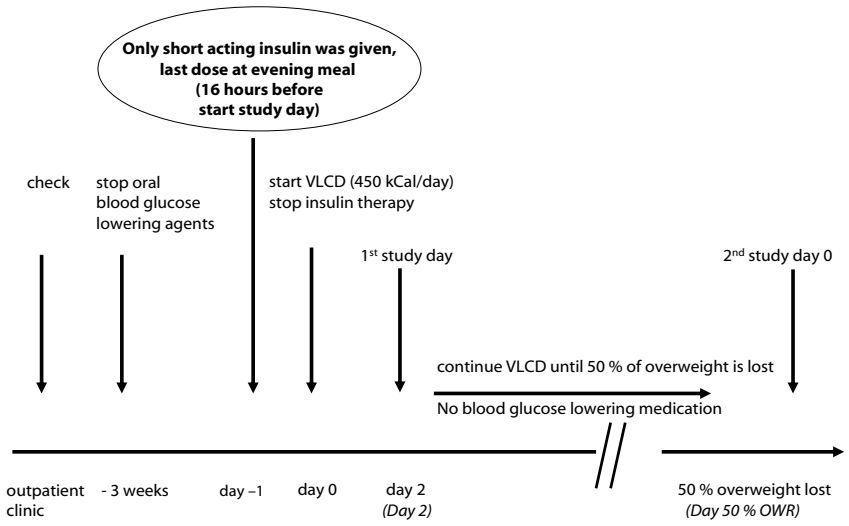

Figure 1
Protocol outline. See text for explanation.

Assessments of body composition

On both study days (day 2 and day 50% OWR), body fat mass (FM) and fat free mass mass (FFM) were measured by Bioelectrical Impedance Analysis (BIA, Bodystat® 1500, Bodystat Ltd., Douglas, Isle of Man,UK). The impedance measurements were performed first thing in the morning after subjects had voided; while they were fasting and resting in bed. On a separate day, close to (1 or 2 days before) day 2 and day 50% OWR, total body fat mass and FFM were also assessed using dual-energy X-ray absorptiometry (Hologic QDR 4500, Hologic, Waltham, MA, USA). The scanner had a coefficient of variation for FM of 2.1% and of 1.0% for LBM. Data obtained for FM and FFM with either technique correlated greatly on both study days ($r = 98$, $p = 0.0001$). Because we did not obtain the correct data in 2 patients on day 50% OWR for the DEXA-scan (only bone mineral density was measured accidentally), we used the data obtained from the BIA for further calculations.

Length (meters [m]) and weight (kilograms [kg]), body mass index (BMI= weight (kg) / length^2 (m)) and waist circumference were measured according to WHO recommendations[10].

Hyperinsulinaemic euglycaemic clamp studies

Metabolic studies were performed as described previously[9]. In short, basal rates of glucose and glycerol turnover were assessed after 3 hours of an adjusted primed (17.6 µmol/kg x actual plasma glucose concentration (mmol/L)/5 (normal plasma glucose)[11] continuous (0.33 µmol/kg per min) infusion of [6,6-$^2H_2$]-glucose (Cambridge Isotopes, enrichment 99.9% Cambridge, USA) and 1.5 hours of a primed (1.6 µmol/kg) continuous (0.11 µmol/kg per min) infusion of [$^2H_5$]-glycerol (Cambridge Isotopes, Cambridge, USA). Subsequently, insulin-stimulated rates of glucose and glycerol turnover were measured after 4.5 hours of a hyperinsulinaemic euglycaemic clamp ((Actrapid®, Novo Nordisk Pharma, Alphen aan de Rijn, The Netherlands; rate 40 mU/m^2/min)[12]. Glucose values were clamped at 5 mmol/L via the infusion of a variable rate of 20% glucose enriched with 3% [6,6-$^2H_2$]-glucose.

Arterialised venous blood samples[13] were collected before the beginning of the tracer infusion, during the last 30 minutes of the basal period (3 times, with 7-minute intervals, *t* = 150-180 minutes after the start of the [6,6-$^2H_2$]-glucose infusion) and during the last 30 minutes of the euglycaemic hyperinsulinaemic clamp (4 times, with 10 minute intervals, *t* = 420-450 minutes). At these time points, blood samples were taken for the determination of [6,6-$^2H_2$]-glucose- and [$^2H_5$]-glycerol-specific activity, glucose, insulin, glycerol, C-peptide, non-esterified fatty acids (NEFAs), triglycerides, lactate, growth hormone (GH), cortisol, glucagon, leptin, resistin and adiponectin.

All blood samples, except serum samples, were immediately put on ice and centrifuged promptly (2000×*g* at 4°C for 20 minutes). Serum samples first had to coagulate before undergoing the same procedure. Samples were subsequently put in plastic tubes and frozen (-20°C) until assay.

At the end of both the basal and the clamp period indirect calorimetry with a ventilated hood (Oxycon Beta, Mijnhardt Jaegher, Breda, The Netherlands) was performed for 30 minutes for the determination of glucose and lipid oxidation rates[14].

Blood chemistry

Serum insulin was measured with an immunoradiometric assay (IRMA, Biosource, Nivelles, Belgium). The detection limit was 3 mU/L and the interassay coefficient of variation was below 6%.

C-peptide, glucagon, leptin, resistin and adiponectin were measured with radioimmuno assays from Linco Research (St. Charles MO, USA). For C-peptide the interassay coefficient of variation (CV) varied between 4.2 and 6.0% at different levels with a sensitivity of 0.03 nmol/L. The CV for glucagon ranged between 4.0 and 6.8% with a sensitivity of 20 ng/L. For leptin the CV was 3.0-5.1% and the sensitivity 0.5 µg/L. For resistin the interassay CV was 3.2- 5.4% at different levels, the lowest detection level was 0.15 µg/L. Adiponectin had an interassay CV of 6.3-8.1% with a lowest detection level of 1 µg/L.

GH was measured with a time-resolved immunofluorescent assay (Wallac, Turku, Finland) specific for the 22 kDa GH. The CV varied from 5.3 to 8.4%, sensitivity 0.03 mU/L. Cortisol was measured with a radioimmunoassay (Sorin Biomedica, Milan, Italy) with CV between 2.3 and 4.2% and a detection limit of 25 nmol/L. Serum triglycerides were measured with a fully automated Modular P 800, serum lactate and fructosamine with a Modular I 800 system, both from Hitachi (Hitachi, Tokyo, Japan) with interassay CVs below 3%.

Serum glucose and [6,6-$^{2}H_{2}$]-glucose as well as serum glycerol and [$^{2}H_{5}$]-glycerol were determined in a single analytical run, using gas chromatography coupled to mass spectrometry as described previously[15,16].

Serum non-esterified fatty acids (NEFA) were measured using the enzymatic colorimetric acyl-CoA synthase/acyl-CoA oxidase assay (Wako Chemicals, Neuss, Germany) with a detection limit of 0.03 mmol/L. The interassay coefficient of variation was below 3%.

Calculations

In all subjects, a physiologic and isotopic steady state was achieved during the last half hour before the clamp (t = 150-180 minutes) and during the last hour of the clamp (t = 390-450 minutes). Therefore, the rate of appearance (R_a) for glucose and glycerol were calculated using Steele's steady-state equation as adapted for stable isotopes using a single-compartment kinetic model[17].

Endogenous glucose production (EGP) during the basal steady state is equal to the R_a of glucose, whereas EGP during the clamp was calculated as the difference between R_a of glucose and the glucose infusion rate.

The hepatic insulin resistance index was calculated as the product of the EGP (µmol.kgFFM^{-1}.min^{-1}) and the plasma insulin concentration (mU/L)[18].

The metabolic clearance rate (MCR) of insulin was calculated as the constant infusion rate of insulin divided by the steady-state serum insulin concentration (SSI). The steady-state insulin concentration was corrected for endogenous insulin secretion using the following formula: SSI = steady-state insulin concentration (basal insulin concentration x [steady state C-peptide/basal C-peptide concentration])[19,20].

Total lipid and carbohydrate oxidation rates were calculated as described by Simonson and DeFronzo[14]. For the conversion of fat oxidation from milligram per kilogram per minute to micromole per kilogram per minute, an average molecular weight of 270 was assumed for serum NEFAs[7]. Non-oxidative glucose metabolism was calculated by subtracting the glucose oxidation rate (determined by indirect calorimetry) from R_d.

Percentage overweight was calculated as 100x(weight/ideal body weight) – 100. Ideal body weight for height was determined according to the Metropolitan Life Insurance tables (1983).

Homeostatic Model Assessment (HOMA) of insulin resistance (IR, normal values approach 1) and β-cell function (% β, 100% is normal) were calculated with the updated computer version (HOMA2) of the formulae of Matthews *et al*[21].

Statistical analysis

Data are presented as mean ± SEM. Differences between day 2 and day 50% OWR were analysed by the Student's *t*-test for paired samples. Non-parametric (Wilcoxon signed-rank test) tests for paired samples were performed when appropriate. All analyses were performed using SPSS for Windows version 12.0 (SPSS Inc., Chicago, IL, USA). Significance was accepted at $p < 0.05$.

RESULTS

Weight and body composition

Weight loss during the first 2 days (day 0 to day 2) amounted –2.1 ± 0.3 kg, reflecting mainly salt and fluid loss. From day 2 until the second study day, patients lost an additional 20.3 ± 2.2 kg ($p = 0.0001$). BMI decreased from 39.7 ± 1.7 on day 2 to 32.3 ± 1.2 kg/m^2 on day 50% OWR ($p = 0.0001$). Mean time to weight loss of 50% of overweight was 17 weeks (range 4-35 weeks).

Body fat mass decreased from 51.0 ± 3.9 kg on day 2 to 32.7 ± 3.0 kg on day 50% OWR ($p = 0.0001$). This indicates that 85% of weight loss was loss of FM, and that LBM was relatively spared. Waist circumference was also reduced significantly (Table 1).

Table 1. Effect of a VLCD on body composition and glycaemic regulation in obese type 2 diabetic patients.

	Before VLCD	Day 2 VLCD	50% reduction of overweight
BMI (kg/m²)	40.2 ± 1.6	39.7 ± 1.7[*]	32.3 ± 1.2[†]
Weight (kg)	113.0 ± 7.1	110.9 ± 6.9[‡]	90.6 ± 5.0[†]
Fat mass (kg)	51.0 ± 3.9	50.1 ± 3.7	32.7 ± 3.0[†]
Waist circumference (cm)	126.8 ± 3.3	126.2 ± 3.5	107.7 ± 3.3[†]
FPG (mmol/L)	11.1 ± 0.8	12.5 ± 0.5	7.8 ± 0.5[†]
Fructosamine (nmol/L)	(HbA_{1c} 7.7 ± 0.4%)	329 ± 11	283 ± 12[§]
Fasting serum insulin (mU/L)	[a]	24.2 ± 2.2	15.2 ± 1.3[‖]
HOMA-IR	[a]	4.1 ± 0.3	2.1 ± 0.2[¶]
HOMA-β	[a]	42.9 ± 4.0	70.9 ± 9.4[#]

Data are presented as mean ± SEM.

[a] values likely to be unreliable because patients had used short-acting insulin therapy until the evening before the start of the VLCD (day 0)

[†] p = 0.0001 compared to both before VLCD and Day 2 VLCD

[§] p = 0.035; [‖] p = 0.001; [¶] p = 0.0001, [#] p = 0.009 day 50% OWR compared to day 2 VLCD.

[‡] p = 0.0001, * p = 0.049 day 2 compared to day 0

Fasting plasma glucose and insulin concentration

FPG levels declined significantly from day 2 of the VLCD until 50% of the overweight was reduced (12.5 ± 0.5 to 7.8 ± 0.5 mmol/L, p = 0.0001). In addition, serum insulin concentrations declined significantly between the two study days from 24.2 ± 2.2 to 15.2 ± 1.3U/L (p = 0.001).

Serum fructosamine levels, a measure for prolonged (2-4 weeks) glucose regulation, declined from 329 ± 11 to 283 ± 12 nmol/L (p = 0.035). HOMA-IR declined significantly whereas HOMA-β increased significantly (Table 1).

Endogenous glucose production and whole-body glucose disposal.

On both study days, serum glucose was clamped at identical levels (5.1 ± 0.3 and 5.4 ± 0.3 mmol/L on day 2 and day 50% OWR respectively, NS). The degree of hyperinsulinaemia was lower on day 50% OWR (80.8 ± 4.0 mU/L) as compared to day 2 (90.2 ± 3.3 mU/L, p = 0.023). This is probably the result of the increased metabolic clearance rate of insulin (see Table 2). The lower clamp serum insulin concentration on day 50% OWR does not negatively affect the results of our study. In fact, at equal and, thus, higher serum insulin levels on day 50% OWR the differences between study days on measures of insulin sensitivity would become even greater.

Basal EGP decreased significantly from day 2 to day 50% OWR (20.0 ± 0.9 and 16.4 ± 1.2 $\mu mol.kgFFM^{-1}.min^{-1}$ on day 2 and day 50% OWR, respectively, p = 0.001, Fig. 2). During the hyperinsulinaemic euglycaemic clamp EGP was significantly lower on day 50% OWR, although the amount of suppression (from basal to clamp) was not significantly different between study days. However, basal and clamp hepatic insulin resistance indexes were significantly lower on day 50% OWR (Table 2).

Table 2. Metabolic parameters during a VLCD on day 2 and after 50% of overweight (50% OWR) was lost, in obese type 2 diabetic patients.

	Day 2	Day 50% OWR	P
Basal EGP$^{\Delta}$	20.0 ± 0.9	16.4 ± 1.2	0.001
Clamp EGP$^{\Delta}$	8.5 ± 0.9*	4.6 ± 1.2*	0.005
Basal HIR$^{\square}$	485 ± 39	249 ± 28	0.0001
Clamp HIR$^{\square}$	756 ± 72	362 ± 91	0.001
Glucose R_d^{Δ}	18.8 ± 2.0	39.1 ± 2.8	0.001
MCR insulin (ml/m^2/min)	0.47 ± 0.02	0.53 ± 0.03	0.028
Basal whole-body glucose oxidation$^{\Delta}$	6.7 ± 1.4	4.2 ± 0.4	NS
Clamp whole-body glucose oxidation$^{\Delta}$	6.1 ± 0.9	12.7 ± 1.5†	0.002
Basal non-oxidative glucose metabolism$^{\Delta}$	14.8 ± 1.1	12.4 ± 1.1	0.036
Clamp non-oxidative glucose metabolism$^{\Delta}$	12.2 ± 1.6	27.7 ± 2.8‡	0.002
Basal glycerol R_a°	16.4 ± 2.3	14.6 ± 1.4	NS
Clamp glycerol R_a°	11.5 ± 2.3	7.5 ± 1.6§	NS
Basal whole-body lipid oxidation$^{\Delta}$	8.0 ± 0.5	7.1 ± 0.5	NS
Clamp whole-body lipid oxidation$^{\Delta}$	8.3 ± 0.3	5.5 ± 0.8$^{\|}$	0.008

Data are presented as mean ± SEM. NS indicates not significant.

$^{\Delta}$ values are in µmol.kgFFM^{-1}.min^{-1} ; $^{\circ}$ values are in µmol.kgFM^{-1}.min^{-1}

$^{\square}$ EGP in µmol.kgFFM^{-1}.min^{-1} was multiplied with plasma insulin in mU/L

$^{*}p = 0.0001$, $^{\dagger}p = 0.001$, $^{\ddagger}p = 0.005$, $^{\S}p = 0.012$, $^{\|}p = 0.011$ clamp versus basal values

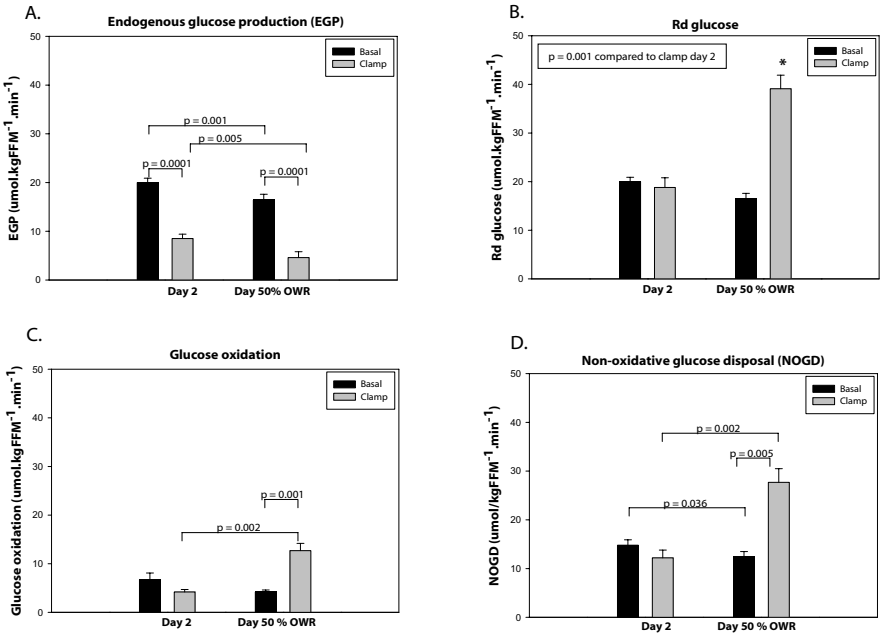

Figure 2.

Endogenous glucose production (EGP) [A], glucose disposal rates (Rd glucose) [B], oxidative [C] and non-oxidative [D] glucose disposal rates in 10 obese type 2 diabetic patients on day 2 of a VLCD and after a weight loss of 50% of the overweight (day 50% OWR). Black bars represent basal values; grey bars represent values during the hyperinsulinaemic euglycaemic clamp. Values are presented as mean ± SEM. Note the decrease in FPG levels and a decrease in basal EGP as well as a better suppression of EGP during insulin stimulation.

Table 3. Effects of weight loss on hormones, substrate levels and adipokines in obese type 2 diabetic patients.

	Day 2	Day 50% OWR	P
Fasting serum cortisol (nmol/L)	451 ± 30	419 ± 34	NS
Fasting serum GH (mU/L)	1.2 ± 0.4	3.7 ± 1.5	0.012
Fasting serum glucagon (ng/L)	63.2 ± 8.3	70.7 ± 5.1	NS
Fasting serum glycerol (µmol/L)	150 ± 15	108 ± 12	0.008
Fasting serum NEFA (mmol/L)	1.6 ± 0.2	1.2 ± 0.1	0.018
Fasting serum triglycerides (mmol/L)	2.7 ± 0.5	1.2 ± 0.1	0.005
Fasting serum leptin (µg/L)	26.9 ± 4.4	11.4 ± 2.8	0.005
Fasting serum resistin (µg/L)	13.3 ± 1.1	11.5 ± 1.0	NS
Fasting serum adiponectin (µg/L)	5.2 ± 0.4	6.6 ± 0.6	0.012
Clamp serum glucose (mmol/L)	5.1 ± 0.3	5.4 ± 0.3	NS
Clamp serum insulin (mU/L)	90.2 ± 3.3	80.8 ± 4.0	0.023
Clamp serum glycerol (µmol/L)	114 ± 18	65 ± 12	0.011
Clamp serum NEFA (mmol/L)	1.1 ± 0.3	0.3 ± 0.1	0.017

Data are presented as mean ± SEM. NS indicates not significant.

Of the hormones involved in the regulation of hepatic glucose production, fasting serum cortisol and glucagon concentrations did not change with weight loss, whereas fasting growth hormone levels (as expected) increased (Table 3).

Insulin stimulated glucose disposal increased from 18.8 ± 2.0 µmol.kgFFM^{-1} on day 2 to 39.1 ± 2.8 µmol.kgFFM^{-1}.min^{-1}on day 50% OWR, p = 0.001 (Fig. 2). This is an increase of 107%.

The MCR of insulin was significantly greater on day 50% OWR, which could explain the lower steady state serum insulin values at the end of the clamp procedure on day 50% OWR (while the insulin infusion rate of 40 mU/m2/min was the same on both study days).

Glycerol R_a and non-esterified fatty acids, glycerol and triglycerides

Basal glycerol R_a decreased from 16.4 ± 2.3 to 14.6 ± 1.4 µmol.kgFM^{-1}.min^{-1} (p = NS) between study occasions. The R_a of glycerol during the clamp was lower on day 50% OWR as compared to day 2, but this difference also did not reach significance. The Glycerol R_a was suppressed to a lower level by insulin on day 50% but if the change in glycerol R_a from basal to hyperinsulinaemia was calculated, statistical significance was not reached (–4.8 ± 2.7 on day 2 *versus* -7.1 ± 2.2 µmol.kgFM^{-1}.min^{-1} after 50% of overweight was lost) (Table 2 and Fig. 3).

However, fasting levels of NEFAs, triglycerides and glycerol declined significantly, and clamp values of serum NEFA and glycerol were also significantly lower at day 50% OWR, reflecting a better suppressibility of lipolysis by insulin (Table 3).

Glucose and lipid oxidation rates

On day 50% OWR, insulin infusion increased the rate of glucose oxidation significantly as compared to day 2. Basal, as well as insulin-stimulated non-oxidative glucose disposal (NOGD) also increased significantly after the weight loss. The capacity of insulin to suppress lipid oxidation was improved with weight loss (Table 2 and Fig. 3).

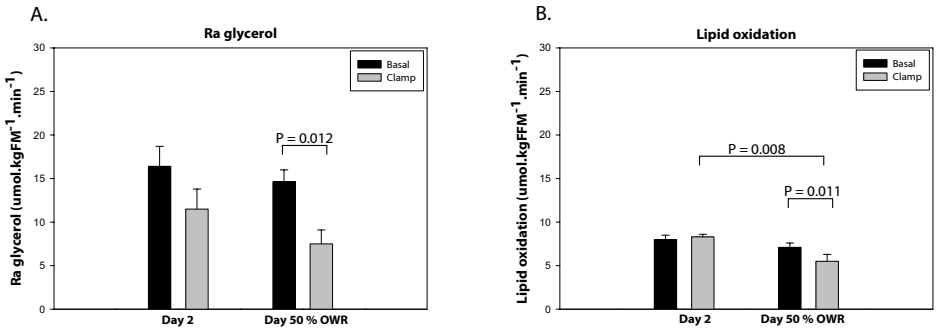

Figure 3.

Glycerol Ra [A] and lipid oxidation [B] rates in 10 obese type 2 diabetic patients on day 2 of a VLCD and after a weight loss of 50% of the overweight (day 50% OWR). Black bars represent basal values, grey bars represent values during the hyperinsulinaemic euglycaemic clamp. Values are presented as mean ± SEM. Note that values for R_a of glycerol are presented in µmol.kgFM^{-1}.min^{-1}, while those for lipid oxidation are in µmol.kgFFM^{-1}.min^{-1}. Weight loss resulted in a decrease in basal whole-body lipolysis and lipid oxidation, with a better suppression during hyperinsulinaemia of both parameters.

Adipokines

As expected with weight loss, serum leptin levels were significantly lower at day 50% OWR. Serum resistin levels were not significantly different between study days but serum adiponectin was significantly higher on day 50% OWR.

DISCUSSION

The aim of the present study was to evaluate the underlying mechanisms by which weight reduction *per se* improves hyperglycaemia in obese insulin-treated type 2 diabetic patients. As compared to caloric restriction *per se* (2-day VLCD[9]), a prolonged VLCD leading to a loss of 50% of overweight led to a substantial improvement in insulin-stimulated glucose disposal, despite the cessation of all blood glucose-lowering medication (including insulin) and the fact that patients were still obese. This improvement in insulin-stimulated glucose uptake was due an improvement in both oxidative and non-oxidative glucose disposal. In addition, insulin sensitivity of the liver and adipose tissue, reflected in the rate of insulin-suppressibility of EGP and lipolysis (R_a glycerol, and hyperinsulinaemic serum FFA and glycerol concentrations), respectively, also improved. This study indicates that prolonged use of a VLCD, resulting in major weight loss, induces additional adaptations in fundamental aspects of glucose metabolism in obese patients with type 2 diabetes mellitus compared to those induced by short-term use of a VLCD.

The increase in insulin-stimulated glucose uptake was due to an increase in both insulin-stimulated glucose oxidation as well as non-oxidative glucose disposal (NOGD). A 2-day VLCD not only had no effect on insulin-stimulated glucose uptake but even decreased NOGD[9]. In

obese and type 2 diabetic patients, total glucose disposal and NOGD during hyperinsulinaemia are much lower compared to controls[22-24]. Since the increase in insulin-stimulated glucose oxidation seems to be bound to a maximum[25], NOGD is quantitatively the most important. Hence the improvement in NOGD is an important finding, indicating that patients were better able to store glucose as glycogen after weight loss. Others found either an increase[5,6,26,27] or no effect[28,29] on NOGD with weight loss following low calorie diets in obese type 2 diabetic[6,27,28,30] patients. The mechanisms underlying an improvement in NOGD are unclear, since several studies failed to demonstrate an effect of weight loss on glycogen synthase activity in skeletal muscle biopsies[26,28,30].

As compared to a 2-day VLCD, basal EGP was reduced further to normal levels. Because we did not measure between day 2 and day 50% OWR we do not know at what time-point normal values for basal EGP were obtained. Given the fact that basal EGP decreased substantially within 2 days of a VLCD[9], and the fact that others found that the greatest reduction in EGP takes place in the first 7-10 days of caloric restriction[2,3] the normalisation of basal EGP probably took place early during the course of the VLCD. The improvement in insulin suppressibility of EGP has been found before[3,6] and occurs already with modest (approximately 8 kg) weight loss[31]. However, Laakso *et al.*[27] did not find an effect of weight loss on insulin sensitivity of the liver. With respect to the causes of the improvement in basal EGP and insulin-suppressibility of EGP, of the hormones we measured, the concentration of glucagon and cortisol did not change while the GH concentration (a hormone known to stimulate EGP) was decreased with weight loss. In addition, the decrease in serum NEFAs and glycerol, and probably also a decrease in intrahepatic fat, might contribute. Furthermore, in rodents and in *in vitro* studies, adiponectin (levels of which were increased with weight loss in our study) can inhibit gluconeogenesis[32,33]. In humans, serum adiponectin levels are negatively correlated with EGP[34].

We found a lower basal and hyperinsulinaemic R_a of glycerol, as well as lower basal and hyperinsulinaemic serum NEFA and glycerol concentrations after weight loss, altogether indicative of a lower basal rate of lipolysis and an improved capacity of insulin to suppress whole-body lipolysis. In healthy and obese humans, short-term fasting increases the basal rate of lipolysis, whereas it remains the same or even decreases following short-term severe caloric restriction in obese type 2 diabetic patients[7,9]. Caloric restriction for a longer period of time in obese patients (VLCD 615 kcal/day during 28 days)[35] and obese patients with type 2 diabetes (10 days 25% of energy requirements and 10 days 75%)[7] has no effect on the basal rate of glycerol R_a. The fact that we found a decline in the basal rate of lipolysis cannot be explained by the lower total body fat mass because we expressed the R_a of glycerol per kilogram fat mass. We presume that the rate of lipolysis has been higher at the beginning of the VLCD, but that with ongoing caloric restriction, because of a lower metabolic rate, utility (lipid oxidation) has decreased, and, because there is a balance between lipolysis (production) and lipid oxidation (utility), lipolysis has also decreased. The cause of the decrease in basal metabolic rate during calorie restriction is unknown but intracellular enzymatic pro-

cesses must be involved. These processes are in themselves regulated by several hormones and the autonomic nervous system. The novelty of our study is that we also documented the effect of a prolonged VLCD leading to substantial weight loss on insulin suppressibility of whole-body lipolysis, measured with [$^2H_5$]-glycerol, in obese insulin-treated obese type 2 diabetic patients, and showed that insulin suppressibility of lipolysis improves with weight loss. We could not compare these results with those of others because data are lacking for this intervention and patient group.

We also documented, with a hyperinsulinaemic euglycaemic clamp technique combined with [6,6-$^2H_2$]-glucose, the magnitude of the improvement in insulin-stimulated glucose disposal (107%) following substantial weight loss in very obese insulin-treated patients with type 2 diabetes. Several studies using the hyperinsulinaemic euglycaemic clamp technique (but without stable isotopes) have been performed in morbidly obese non-diabetic patients before and after substantial weight loss following bariatric surgery. M-values in the lean control groups in these studies were around 50 µmol.kg LBM^{-1}.min^{-1} (LBM = lean body mass)[36-38]. After significant weight loss (50-60 kg) M-values in obese patients increased from 7-19 µmol.kg LBM^{-1}.min^{-1} to around 35 µmol.kg LBM^{-1}.min^{-1} in 2 studies[36,39] and even above 50 µmol.kg LBM^{-1}.min^{-1} in 2 other studies[37,38], while their BMI remained in the obese range after weight loss (30-39.9 kg/m^2), like in our patients. When we calculated M-values in our study, patients increased from 9.9 ± 2.3 to 37.2 ± 4.6 µmol.kg LBM^{-1}.min^{-1}. Although the effectiveness of bariatric surgery in improving type 2 diabetes has been established in several studies[40-42] (review in[43]), unfortunately again no data on glucose disposal rates are available in obese type 2 diabetic patients.

Hence, short-term energy, or, more likely, carbohydrate restriction, improves hyperglycaemia primarily via a reduction in basal EGP[9,44]. Modest weight loss (approximately 8 kg) also improves hepatic insulin sensitivity[31], and substantial weight loss improves all aspects of glucose metabolism (this study). Given the fact that weight loss induced by subcutaneous liposuction does not lead to an improvement in insulin sensitivity (and adipokines such as leptin and adiponectin)[45], whereas weight loss with a decrease in waist circumference (like we found) does, indicates a role for energy restriction and/or upper body obesity (i.e., visceral adipose tissue and/or the deep layers of abdominal subcutaneous tissue). Unfortunately, we did not measure visceral fat mass and hence could not investigate whether the improvement in glucose and lipid metabolism we found, is correlated with a decrease in visceral fat mass. The decline in fasting as well as clamp levels of NEFA and triglycerides suggests a decrease in lipotoxicity.

In conclusion, prolonged caloric restriction leading to 50% reduction of overweight in obese type 2 diabetic patients simultaneously taken off all blood glucose-lowering medication (including insulin), considerably improves insulin sensitivity of endogenous glucose production, peripheral glucose uptake and lipolysis, even though patients were still obese (BMI 32.3 ± 1.2 kg/m^2). These observations stress that weight-reducing strategies, especially diets, should be a cornerstone of therapy in obese type 2 diabetic patients.

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