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

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Citation

Jazet, I. M. (2006, April 11). *Insulin resistance in obese patients with type 2 diabetes mellitus : effects of a very low calorie diet*. Retrieved from <https://hdl.handle.net/1887/4366>

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CHAPTER 5

Two days of a very low calorie diet reduces endogenous glucose production in obese type 2 diabetic patients despite the withdrawal of blood glucose-lowering therapies, including insulin.

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Metabolism 2005;54(6):705-12

ABSTRACT

The mechanism of the blood glucose-lowering effect of a 2-day very low calorie diet (VLCD; 1890 kJ/d [450 kCal/day]) in combination with the cessation of all blood glucose-lowering agents was studied in 12 (7 women, 5 men) obese (body mass index 36.3 ± 1.0 kg/m² [mean \pm SEM]) type 2 diabetic patients (age 55 ± 4 years; HbA_{1c} $7.3 \pm 0.4\%$) undergoing insulin therapy. Endogenous glucose production (EGP) and whole-body glucose disposal ([6,6-²H₂]-glucose), lipolysis ([²H₃]-glycerol), and substrate oxidation (indirect calorimetry) rates were measured before and after the intervention in basal and hyperinsulinaemic euglycaemic conditions.

After 2 days of a VLCD and discontinuation of all blood glucose-lowering therapies, fasting plasma glucose levels did not increase (11.3 ± 1.3 versus 10.3 ± 1.0 mmol/L). Basal EGP significantly declined (14.2 ± 1.0 to 11.9 ± 0.7 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $p = 0.009$). Basal metabolic clearance rate of glucose and rate of basal lipolysis did not change. During hyperinsulinaemia, EGP (5.5 ± 0.8 to 5.2 ± 0.5 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), whole-body glucose disposal (12.1 ± 0.7 to 11.3 ± 1.0 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), the metabolic clearance rate of glucose, and the rate of lipolysis did not change after the 2-day intervention.

In conclusion, cessation of blood glucose-lowering therapy in combination with a 2-day VLCD does not lead to hyperglycaemia and is associated with a reduction in basal EGP. Insulin-stimulated whole-body glucose disposal did not improve, nor did insulin suppressibility of EGP and lipolysis.

INTRODUCTION

There is a strong relationship between type 2 diabetes and obesity¹, more than 70% of type 2 diabetic patients are overweight and obese². In obese patients, insulin resistance is the most important underlying defect leading to glucose intolerance and, subsequently, when hyperinsulinaemia is insufficient to overcome insulin resistance, type 2 diabetes develops³. Numerous studies have shown that weight loss diminishes the metabolic abnormalities of obese type 2 diabetic patients⁴⁻¹⁰. Because patients usually find it difficult to adhere to a diet, very low calorie diets (VLCDs) have been advocated. The rapid weight loss achieved with these diets is an important stimulus for patients to continue. The simultaneous discontinuation of a blood glucose-lowering therapy facilitates weight loss and minimises the risk of hypoglycaemia but raises concern about possible hyperglycaemia. We recently showed in a group of obese type 2 diabetic patients, in whom we discontinued all blood glucose-lowering therapies including insulin, that a VLCD (Modifast®; 450 kCal/day) does not lead to a deterioration of fasting plasma glucose (FPG) levels¹¹. In fact, in most patients, a decrease in FPG occurred already after 2 days of the VLCD, when weight loss was minimal.

A decline in FPG levels before significant weight loss occurred has been described before^{5,6,9,12}. Several studies have shown that FPG declined in parallel with hepatic glucose output^{5,6,8,12}. However, to our knowledge, no one has studied this effect in detail after only 2 days of a VLCD. In addition, few studies address the patient group we are interested in: severely obese type 2 diabetic patients inadequately regulated on insulin therapy. We therefore studied obese type 2 diabetic patients undergoing insulin therapy with or without oral blood glucose-lowering agents before and after 2 days of a VLCD in combination with the cessation of these medications.

We used the isotope dilution technique to measure endogenous glucose production (EGP) in combination with the hyperinsulinaemic euglycaemic clamp technique to study insulin-mediated peripheral glucose disposal and insulin suppressibility of EGP. In addition, we measured total-body lipolysis *via* the infusion of deuterium-labelled glycerol and substrate oxidation rates *via* indirect calorimetry.

RESEARCH DESIGN AND METHODS

Subjects

A total of 12 obese type 2 diabetic patients, 5 men and 7 women with a mean age of 55 ± 4 years (mean \pm SEM) and a body mass index (BMI) of 36.3 ± 1.0 kg/m² (range 31.3 – 43.9 kg/m²), participated in this study, which was approved by the Medical Ethical Committee of the Leiden University Medical Centre. Written informed consent was obtained from all patients. Patients underwent a medical screening including a physical examination and resting

electrocardiogram. Patients used at least 30 units of exogenous insulin with or without oral blood glucose-lowering medication and had a BMI > 30 kg/m². In addition, they had to have remaining endogenous insulin secretion defined as a fasting plasma C-peptide level greater than 0.8 ng/mL or a 2-times increase of the basal C-peptide level after 1 mg glucagon i.v.¹³.

Patients had to have a stable 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 any other medication (than that used specifically for its glucose-lowering effect) known to alter glucose or lipid metabolism was prohibited.

Protocol

Three weeks before the start of the study, all oral blood glucose-lowering medication was discontinued. On day -1, only short-acting insulin was given, evening doses of intermediate and long-acting insulin were omitted. On day 0, patients were admitted to the research centre for baseline investigations (day 0) as outlined below. Insulin therapy was restarted after this study day until the start of the VLCD (again, only short-acting insulin was given on the day before the start of the diet) and remained stopped during the 2-day VLCD. To ensure complete washout of the stable isotopes, the second study had to be undertaken 1 week later. This meant that patients started the 2-day VLCD (1890 kJ/d) on day 5 and had the second study on day 7 (day 2). (See Fig. 1)

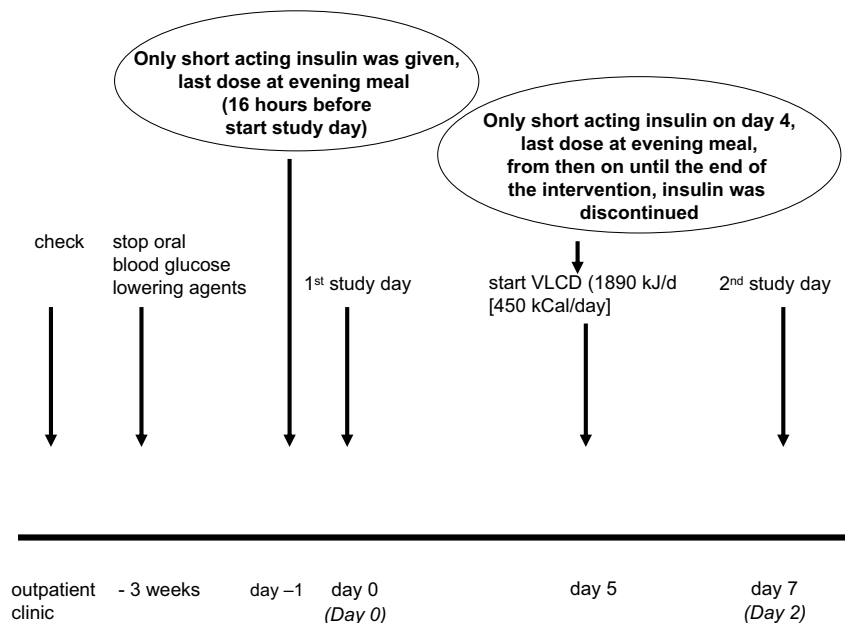


Figure 1.

Protocol outline. See text (methods) for explanation.

STUDY DAYS

All studies started at 7:00 AM after an overnight fast. Length (meters [m]), weight (kilograms [kg]), BMI (weight [kg] / length² [m]) and waist-hip circumference were measured according to WHO recommendations¹⁴.

Patients were subsequently requested to lie down on a bed in a semirecumbent position. A polyethylene catheter was inserted into an antecubital vein for infusion of test substances. Another catheter was inserted into a contralateral dorsal hand vein for blood sampling. This hand was kept in a heated box (60°C) throughout the test to obtain arterialised venous blood samples¹⁵. Basal blood samples for glucose, insulin, C-peptide, non-esterified fatty acids (NEFAs), glycerol, and background enrichment of [6,6-²H₂]-glucose and [²H₅]-glycerol were taken. At 7:30 AM (*t* = 0 minutes), an adjusted primed (17.6 μmol/kg x actual plasma glucose concentration (mmol/L)/5(normal plasma glucose)¹⁶ continuous (0.33 μmol/kg per minute) infusion of [6,6-²H₂]-glucose (enrichment 99.9%; Cambridge Isotopes, Cambridge, Mass, USA) was started and continued throughout the study. At 9:00 AM (*t* = 90 minutes) a primed (1.6 μmol/kg) continuous (0.11 μmol/kg per minute) infusion of [²H₅]-glycerol (Cambridge Isotopes) was started and continued throughout the study. During this period, indirect calorimetry with a ventilated hood (Oxycon Beta, Mijnhardt Jaegher, Breda, The Netherlands) was performed for 30 minutes for basal glucose and lipid oxidation rates¹⁷. At the end of the basal period, 3 blood samples were taken at 7-minute intervals for the determination of plasma glucose, glycerol, insulin, and [6,6-²H₂]-glucose- and [²H₅]-glycerol-specific activities. In addition, blood samples for the determination of NEFAs, triglycerides, lactate, the counterregulatory hormones (growth hormone [GH], cortisol, and glucagon), as well as some of the adipokines involved in glucose metabolism (leptin, resistin and adiponectin) were taken. Subsequently, a primed continuous infusion of insulin (Actrapid®, Novo Nordisk Pharma, The Netherlands, 40 mU/m² per minute)¹⁸ was started (*t* = 180 minutes). Exogenous glucose 20% enriched with 3% [6,6-²H₂]-glucose was infused at a variable rate to maintain the plasma glucose level at 5.0 mmol/L. A second indirect calorimetry was performed at the end of the hyperinsulinaemic clamp (*t* = 390 minutes). From *t* = 420 to 450 minutes, blood was drawn every 10 minutes for the determination of [6,6-²H₂]-glucose- and [²H₅]-glycerol-specific activity, glucose, insulin, glycerol, C-peptide, NEFAs, triglycerides, lactate, 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.

BLOOD CHEMISTRY

Serum insulin, C-peptide, glucagon, GH, cortisol, leptin, resistin, adiponectin, triglycerides, and lactate were measured in one batch. Serum insulin was measured with an ultrasensitive Human Insulin assay (Linco Research, St Charles, MO) with a detection limit of 0.1 mU/L. The interassay coefficient of variation (CV) was below 6%.

C-peptide, glucagon, leptin, resistin and adiponectin were measured with radioimmunoassays from Linco Research. 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% to 5.1% and the sensitivity was 0.5 µg/L. For resistin, the interassay CV was 3.2% to 5.4% at different levels, with the lowest detection level of 0.15 µg/L. Adiponectin had an interassay CV of 6.3% to 8.1% with the lowest detection level of 1 µg/L.

Growth hormone was measured with a time-resolved immunofluorescent assay (Wallace Inc, Turku, Finland) specific for the 22-kDa GH. The CV varied from 5.3% to 8.4%, sensitivity was 0.03 mU/L. Cortisol was also measured with a radioimmunoassay (Sorin Biomedica, Milan, Italy) with a CV between 2.3% and 4.2% and a detection limit of 25 nmol/L. Serum triglycerides and lactate were determined with a fully automated Hitachi 747 system (Hitachi, Tokyo, Japan).

Serum glucose and [6,6-²H₂]-glucose as well as serum glycerol and [²H₅]-glycerol were determined in a single analytical run, using gas chromatography coupled to mass spectrometry as described previously^{19,20}.

Serum non-esterified fatty acids 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%.

Very low calorie diet

The diet consisted of 3 sachets of Modifast® (Novartis Consumer Health, Breda, The Netherlands) per day. Modifast® is a commercially available VLCD packaged in powder form. One sachet is mingled with 250 mL of water and is used to replace each of the 3 conventional meals. We provided patients with shakes, muesli, pudding and potage in various tastes. One hundred grams of Modifast® contains 1402.8 kJ [334] kcal and about 35 g protein, 6 g fat and 38 g carbohydrates. Since sachets vary from 42 to 50 gram, energy intake could range from 1764 to 2062.2 kJ/d depending on the products used. Patients were allowed to drink calorie-free substances *ad libitum* and were encouraged to drink at least 2 L of these liquids per day.

Calculations

In all subjects, both plasma glucose concentrations and tracer/tracee ratios of [6,6-²H₂]-glucose and [²H₅]-glycerol were stable during the last half hour before the clamp ($t = 150-180$

minutes) and during the last hour of the clamp ($t = 390-450$ minutes). In addition, the plasma glucose concentration did not decline during the last hour before the clamp and the last hour of the euglycaemic clamp. 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 model²¹.

Endogenous glucose production during the basal steady state is equal to the R_a of [6,6-²H₂]-glucose, whereas endogenous glucose production during the clamp was calculated as the difference between R_a and the glucose infusion rate.

The metabolic clearance rate (MCR) of glucose was calculated as the rate of disappearance of glucose (R_d ; identical to R_a under steady-state conditions) divided by the serum glucose concentration (average of steady-state measurements at $t = 150-180$ and $t = 420-450$ minutes, respectively).

Total lipid and carbohydrate oxidation rates were calculated as described by Simonson and DeFronzo¹⁷. 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 NEFAs¹². Non-oxidative glucose metabolism was calculated by subtracting the glucose oxidation rate (determined by indirect calorimetry) from R_d .

Statistical analysis

Data are presented as mean \pm SEM unless stated otherwise. Differences before (day 0) and after (day 2) the VLCD were analysed by the Student *t*-test for paired samples. Correlation analysis was carried out using Pearson's correlation. All analyses were performed using SPSS for Windows version 11.0 (SPSS Inc, Chicago, IL, USA). Significance was accepted at $p < 0.05$.

RESULTS

Of the 12 patients participating in this study, clamp data from one female patient had to be excluded from the analysis because of errors in the infusion rate in the afternoon of the second study day. Basal data from this patient and substrate oxidation rates could be and were used, however. Patient characteristics can be found in Table 1.

Weight

After 2 days of a VLCD, patients had lost -2.9 ± 0.4 kg. Presumably, this weight loss reflects mostly salt and fluid loss.

Table 1. Patient characteristics.

Sex (male/female)	5	:	7
Age (years)	55	±	4
BMI (kg/m ²)	36.3	±	1.0
Waist circumference (cm)	120	±	3
Waist-hip ratio	1.02	±	0.03
Fasting plasma glucose (mmol/L)	11.3	±	1.3
HbA _{1c} (%)	7.3	±	0.4
Fasting serum insulin (mU/L)	20.7	±	2.1
Fasting serum C-peptide (ng/mL)	1.0	±	0.1
Duration of type 2 diabetes (years)	7.9	±	1.3
Units of insulin injected per day	78	±	9
Additional use of oral glucose-lowering medication	6 metformin		1 rosiglitazone

Data are presented as mean ± SEM.

Fasting plasma glucose and insulin concentration

After 2 days of a VLCD, despite minimal weight loss (see above) and the cessation of all blood glucose-lowering agents, FPG did not increase. Basal serum insulin levels declined from 20.7 ± 2.3 to 15.9 ± 1.8 mU/L ($p = 0.033$) (Table 2).

Endogenous glucose production, whole-body glucose disposal, and MCR of glucose

Basal EGP declined from 14.2 ± 1.0 to 11.9 ± 0.7 mmol/L ($p = 0.008$). On both study days, serum glucose was clamped at identical levels (5.0 ± 0.4 mmol/L on day 0 and 4.9 ± 0.4 mmol/L on day 2, $p = \text{NS}$) and the same degree of hyperinsulinaemia was obtained (88.1 ± 5.9 mU/L on day 0 and 83.7 ± 4.8 mU/L on day 2, $p = \text{NS}$) (see also Table 2). Insulin decreased EGP (from 14.2 ± 1.0 to 5.5 ± 0.8 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ on day 0) but could not completely suppress it. A 2-day

Table 2. Metabolic parameters at baseline (day 0) and after 2 days of a VLCD (day 2) in obese type 2 diabetic patients.

	Day 0	Day 2	P
Fasting serum glucose (mmol/L)	11.3 ± 1.3	10.3 ± 1.0	NS
Fasting serum insulin (mU/L)	20.7 ± 2.3	15.9 ± 1.8	0.033
Fasting serum cortisol (nmol/L)	570 ± 69	612 ± 58	NS
Fasting serum GH (mU/L)	1.9 ± 0.9	1.2 ± 0.4	NS
Fasting serum glucagon (ng/L)	57.3 ± 7.7	64.2 ± 8.6	NS
Fasting serum glycerol ($\mu\text{mol/L}$)	137 ± 19	186 ± 32	NS
Fasting serum NEFA (mmol/L)	1.1 ± 0.1	1.5 ± 0.1	NS
Fasting serum triglycerides (mmol/L)	1.8 ± 0.2	2.0 ± 0.2	NS
Fasting serum lactate (mmol/L)	0.9 ± 0.1	0.8 ± 0.04	NS
Clamp serum glucose (mmol/L)	5.0 ± 0.4	4.9 ± 0.4	NS
Clamp serum insulin (mU/L)	88.1 ± 5.9	83.7 ± 4.8	NS
Clamp serum glycerol ($\mu\text{mol/L}$)	60.0 ± 6.2	56.3 ± 7.0	NS
Clamp serum NEFA (mmol/L)	0.39 ± 0.07	0.35 ± 0.04	NS

Values are presented as mean ± SEM.

NS indicates not significant.

Table 3. Metabolic parameters at baseline (day 0) and after 2 days of a VLCD (day 2) in obese type 2 diabetic patients.

	Day 0	Day 2	P
Basal EGP ^a	14.2 ± 1.0	11.9 ± 0.7	0.008
Clamp glucose R _a = R _d	12.1 ± 0.7	11.3 ± 1.0	NS
Clamp EGP	5.5 ± 0.8 [*]	5.2 ± 0.5 [*]	NS
Basal MCR	1.5 ± 0.1	1.4 ± 0.1	NS
Clamp MCR	2.6 ± 0.2 [*]	2.4 ± 0.3 [*]	NS
Basal whole-body glucose oxidation	6.1 ± 0.8	3.0 ± 0.4	0.0001
Clamp whole-body glucose oxidation	8.8 ± 1.0 [†]	6.4 ± 0.6 [*]	0.015
Basal non-oxidative glucose metabolism	8.6 ± 1.0	8.9 ± 0.7	NS
Clamp non-oxidative glucose metabolism	3.0 ± 1.3 [‡]	5.2 ± 1.0 [‡]	NS
Basal glycerol R _g	5.2 ± 1.0	4.0 ± 0.6	NS
Clamp glycerol R _g	1.9 ± 0.2 [‡]	1.8 ± 0.2 [‡]	NS
Basal whole-body lipid oxidation	3.8 ± 0.2	4.5 ± 0.1	0.002
Clamp whole-body lipid oxidation	2.9 ± 0.2 [*]	3.4 ± 0.2 [*]	0.022

All values are presented as mean ± SEM. ^aUnits are in $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$.

Clamp compared to basal values: ^{*}p = 0.0001; [†]p = 0.001; [‡]p < 0.008

R_a = glucose rate of appearance, R_d = glucose rate of disappearance, MCR = metabolic clearance rate of glucose

VLCD showed no improvement of insulin suppressibility of EGP (see also Table 3). Glucose Rd did not increase during hyperinsulinaemia on both day 0 and day 2, indicating that patients remained severely insulin resistant. Serum glucose MCR, both basal as well as during hyperinsulinaemia, also did not reveal any significant change between study days (Table 3, Fig. 2).

Non-esterified fatty acids, lactate, glycerol, triglycerides, and hormones

Basal plasma NEFA levels increased from 1.1 ± 0.1 to 1.5 ± 0.1 mmol/L after 2 days of a VLCD (p = NS). Plasma NEFAs were suppressed during the hyperinsulinaemic euglycaemic clamp to 0.4 ± 0.06 and 0.4 ± 0.04 on day 0 and day 2, respectively (change between study days, NS). Basal and hyperinsulinaemic glycerol, triglyceride, and lactate levels did not significantly change after a 2-day VLCD as well (Table 2).

We also measured the serum concentrations of the counterregulatory hormones: glucagon, cortisol and GH. None of these hormones showed significant changes between day 0 and day 2 in either the basal or insulin-stimulated state.

Basal serum leptin levels showed a significant decline after a 2-day VLCD. Only serum leptin levels showed a significant correlation with BMI ($r = 0.73$, $p = 0.007$ on day 0; $r = 0.81$, $p = 0.001$ on day 2). None of the 3 adipokines (leptin, resistin, and adiponectin) showed (before and after the intervention) a correlation with measures of insulin resistance such as fasting serum insulin, MCR and R_d of glucose (data not shown).

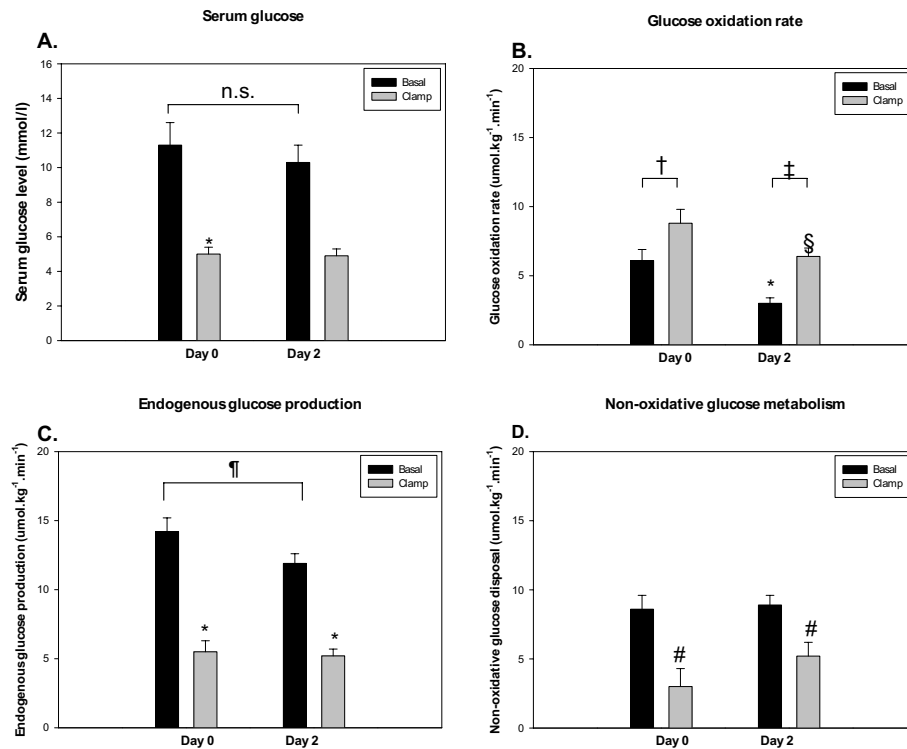


Figure 2.

Plasma glucose levels (A), endogenous glucose production (C) and oxidative (B) and non-oxidative (D) glucose disposal in 12 obese type 2 diabetic patients before and after a 2-day VLCD. Black bars represent basal values, grey bars represent values during the hyperinsulinaemic clamp. Values are presented as mean \pm SEM. Note the decrease in FPG (A, black bars) due to a decrease in basal EGP (C, black bars), and the switch from glucose (B) to lipid oxidation (D).

Clamp compared with basal: * $p = 0.0001$; † $p < 0.008$; ‡ $p = 0.015$

Day 0 compared with day 2: † $p = 0.001$; ‡ $p = 0.0001$; § $p = 0.008$

n.s. indicates not significant.

Glycerol R_a

Basal glycerol R_a did not change significantly after a 2-day VLCD. Insulin significantly suppressed glycerol R_a (5.2 ± 1.0 to 1.9 ± 0.2 $\mu\text{mol.kg}^{-1}.\text{min}^{-1}$ on day 0 [$p = 0.004$] and from 4.0 ± 0.6 to 1.8 ± 0.2 $\mu\text{mol.kg}^{-1}.\text{min}^{-1}$ on day 2 [$p = 0.002$]). Glycerol R_a during hyperinsulinaemia was not different between study days (Table 3).

Glucose and lipid oxidation rates

Both basal and insulin-stimulated glucose oxidation rates significantly decreased after a 2-day VLCD, whereas lipid oxidation rates (both basal and insulin stimulated) increased. Basal as well as clamp non-oxidative glucose disposal remained the same before and after the 2-day VLCD (Table 3).

DISCUSSION

In this study, we assessed the determinants of the blood-glucose lowering effect of 2 days of energy restriction (VLCD; 1890 kJ/d [450 kCal/day]) in severely obese type 2 diabetic patients in whom all blood glucose-lowering agents including insulin were discontinued.

In the absence of a deterioration of blood glucose levels, we demonstrated a decrease of basal EGP. Insulin-stimulated whole-body glucose disposal did not improve, nor did insulin suppressibility of EGP and lipolysis.

Several studies have proven that energy restriction leads to a reduction in FPG levels⁴⁻¹⁰ and even that FPG is closely and positively correlated to basal EGP^{5,6,8}. However, these studies were either incapable of distinguishing between the effects of energy restriction and those of weight loss on glucose metabolism or were performed in a patient group with mild type 2 diabetes. Only one study¹² closely matches our study with regard to patient population (i.e., severely obese type 2 diabetic patients undergoing insulin therapy) and timing of the first study day (although still on day 5, in comparison with day 2 in our study). However, their patients were probably provided with more calories compared with our patients, who received on average 1890 kJ/d [450 kCal/d]. In addition, it is not clear how much insulin the patients in the Christiansen *et al.* study used. Given that oral glucose-lowering medication and/or insulin were discontinued 2 weeks before the start of the study with no major dysregulation of their blood sugar levels despite the fact that they still ate their usual amount of calories suggests that these patients used little medication and had milder diabetes than did our patients. Nonetheless, in the study of Christiansen *et al.*, the short period of energy restriction also led to a decrease in FPG levels caused by a reduction in basal EGP. Remarkably, the reduction in EGP was entirely caused by a decrease in glycogenolysis.

We only measured total EGP and could not discriminate between gluconeogenesis and glycogenolysis. The finding of Christiansen *et al.*¹² that a decreased glycogenolysis accounts for the decline in EGP after energy restriction is further supported by Clore *et al.*²² and Clore and Blackard²³. They repeatedly show that liver glycogen stores are preserved in type 2 diabetic patients after a 3-day fast, suggesting that glycogenolysis is suppressed. However, another study investigated type 2 diabetic patients and control subjects between 14 and 22 hours of fasting²⁴. In that study, both gluconeogenesis and glycogenolysis declined during the fast, with a greater reduction of gluconeogenesis in diabetic subjects compared with control subjects. We believe that a decrease in glycogenolysis would be more obvious because higher doses of insulin are needed to suppress gluconeogenesis as compared to glycogenolysis^{25,26}. So, we postulate that, in our study, the decreased basal EGP can be ascribed to a decrease in glycogenolysis, particularly because the decrease in basal EGP occurred despite lower basal serum insulin levels on day 2. This would suggest that the liver, in the postabsorptive state, has become more sensitive to insulin, at least with respect to glycogenolysis. However, 2 days of energy restriction had no effect whatsoever on insulin's capacity to suppress EGP dur-

ing the hyperinsulinaemic clamp. This inability to demonstrate an effect of 2 days of energy restriction on insulin action in the liver (and in adipose tissue) may have been caused by the relatively high insulin levels (88 mU/L [528 pmol/L] and 84 mU/L [504 pmol/L] on day 0 and day 2, respectively) achieved during the clamp. These concentrations might have been high enough for a near-maximal suppression of the glucose and glycerol R_a . Perhaps a differentiating effect between the 2 study days would be found if glucose and glycerol R_a were studied at lower insulin concentrations.

Basal EGP showed a significant decrease of 16% after 2 days of a VLCD whereas basal FPG levels decreased only by 8%. Normally, a close correlation is found between FPG and basal EGP^{5,27}. Our patient group, however, had higher FPG levels than that in the study of Fery²⁷ and the number of patients we studied was much smaller than that of Henry *et al.*⁵, who also pooled the data of 4 time point measurements from each patient (giving 58 measurements). Hence, one possible explanation for the discrepancy between the results from our study and those from other studies^{5,27} regarding the relation between EGP and FPG could be the small sample size in our study. On the other hand, although the change was not significant, FPG levels did decrease and, hence, the substrate-driven glucose uptake could have decreased after 2 days of a VLCD (clamp glucose disposal tended to decrease on day 2; see Table 3), which might have partly counteracted the decrease in EGP levels.

Another finding of this study was a lack of improvement in whole-body glucose disposal and glucose MCR. This is also in accordance with the study of Christiansen *et al.*¹². They found an increase in MCR not before day 20 of energy restriction. In patients with mild diabetes (undergoing a diet or oral blood glucose-lowering medication only) a 4-day energy-restricted diet (but still providing 4620 +/- 1050 kJ/d [1100 ± 250 kCal/day]) even resulted in a deterioration of basal MCR of glucose and of insulin-stimulated glucose disposal⁹. The latter is in accordance with fasting^{28,29} and low caloric feeding³⁰ studies in lean normal glucose-tolerant subjects who show a decreased peripheral glucose disposal as well. From an evolutionary perspective, this is understandable since more glucose will now be available for the brain. The fact that this response is not apparent in obese type 2 diabetic patients is probably the result of the already severely insulin-resistant state.

The fact that NOGD decreased during the hyperinsulinaemic euglycaemic clamp was unexpected. In healthy subjects, NOGD increases, along with total glucose disposal during hyperinsulinaemia, whereas the rate of increase in glucose oxidation seems to be bound to a limit³¹, indicating that NOGD is quantitatively the most important. In obese and type 2 diabetic patients, NOGD is disturbed. With increasing obesity and insulin resistance, total glucose disposal and NOGD during hyperinsulinaemia are much lower compared with control subjects^{32,33}. Our patients had severe insulin resistance. Despite clamp insulin levels of 88 and 83 mU/L on day 0 and day 2 respectively, glucose disposal did not change significantly and NOGD decreased. There was apparently some room for a slight increase in glucose oxidation

during hyperinsulinaemia. These findings reflect the severely insulin-resistant state of our subjects with a core defect in glucose storage as glycogen (NOGD).

We showed, in accordance with Markovic *et al.*⁹ and Christiansen *et al.*¹², a switch from carbohydrate to lipid oxidation. What we had not expected beforehand was that the rate of basal lipolysis did not increase. This is in contrast to data found in lean nondiabetic subjects who show an increase in whole-body glycerol turnover and whole-body lipid oxidation after 5 days of energy restriction³⁴. However, 2 other studies in obese³⁵ and obese diabetic¹² patients (albeit performed after a longer period of energy restriction [5-20 days]), also found no increase in basal lipolysis. This might be indicative of a disturbed lipid metabolism in obese and obese diabetic subjects. On the other hand, the R_a of glycerol might have been already maximally elevated in these insulin resistant subjects, leaving no room for further increment of lipolysis during fasting. The increased lipid oxidation might therefore be counterbalanced by a decrease in lipogenesis.

We found no arguments for a role of the counterregulatory hormones we measured in the blood glucose-lowering effect of the VLCD because the concentrations of these hormones remained unchanged. This is also true for the adipokines adiponectin and resistin. Whereas the role of resistin in insulin resistance in human beings is controversial³⁶, it is well established that adiponectin concentrations are negatively correlated with insulin resistance, even independently of BMI^{37,38}. Adiponectin levels increase with weight loss in parallel with insulin sensitivity³⁹. We found no change in serum adiponectin levels after 2 days of a VLCD, which is consistent with the fact that we also found no change in insulin sensitivity and only a small amount of weight loss, mainly reflecting salt and fluid loss. Leptin, another adipocyte-derived hormone has a major role in maintaining energy homeostasis but is also thought to have glucose- and insulin-lowering properties^{40,41}. The decrease in serum leptin levels we found most likely reflects the negative energy balance and is consistent with findings in other studies.

We were particularly interested in obese type 2 diabetic patients undergoing insulin therapy because adequately regulated blood glucose levels are usually not achieved in these patients, instead, insulin usually aggravates the insulin-resistant state by inducing weight gain. The fact that plasma glucose levels do not deteriorate despite the cessation of all blood glucose-lowering agents offers therapeutic options. The current study was designed to study the mechanism underlying the early reduction in blood glucose levels after energy restriction and not its long-term effect. We observed, however, that 2 patients had increasing blood glucose levels during the first few days of the VLCD but ended up normoglycaemic (without any form of medication) after continuation of this diet and substantial weight loss. We are currently investigating the effect on glucose metabolism of short-term energy restriction *versus* longer-term energy restriction with substantial weight loss, again in obese type 2 diabetic patients undergoing insulin therapy. Further studies are warranted to determine if any factor can predict *a priori* which patients will benefit from the diet on the long term. This might withhold doctors to treat potentially nonresponsive patients with a demanding VLCD.

In conclusion, despite the cessation of large doses of insulin and oral blood glucose-lowering medication in obese type 2 diabetic patients, FPG levels do not increase and even tend to decline already after 2 days of a VLCD, when weight loss is minimal. The mechanism underlying this early effect of a VLCD is a reduction in basal EGP and not an improvement in whole-body glucose disposal.

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