Effects of Insulin Treatment in Type 2 Diabetic Patients on Intracellular Lipid Content in Liver and Skeletal Muscle

Christian Anderwald, Elisabeth Bernroider, Martin Krššák, Harald Stingl, Attila Brehm, Martin G. Bischof, Peter Nowotny, Michael Roden, and Werner Waldhäusl

Insulin resistance is frequently associated with increased lipid content in muscle and liver. Insulin excess stimulates tissue lipid accumulation. To examine the effects of insulin and improved glycemia on insulin sensitivity and intracellular lipids, we performed stepped (1, 2, and 4 mU · min^{-1} · kg^{-1}) hyperinsulinemic-euglycemic clamps in eight type 2 diabetic and six nondiabetic control subjects at baseline and after 12 and 67 h of insulin-mediated near-normoglycemia (118 ± 7 mg/dl). Intrahepatocellular lipids (IHCLs) and intramyocellular lipids (IMCLs) of soleus (IMCL-S) and tibialis anterior muscle (IMCL-TA) were measured with ^{1}H nuclear magnetic resonance spectroscopy. At baseline, nondiabetic subjects had an approximate twofold higher insulin sensitivity ($P < 0.02$) and lower IHCLs than diabetic patients (5.8 ± 1.2 vs. 18.3 ± 4.2%, $P < 0.03$), in whom IMCL-TA negatively correlated with insulin sensitivity ($r = -0.969$, $P < 0.001$). After a 67-h insulin infusion in diabetic patients, IMCL-S and IHCLs were increased ($P < 0.05$) by ~36 and ~18%, respectively, and correlated positively with insulin sensitivity (IMCL-S: $r = 0.982$, $P < 0.0005$; IHCL: $r = 0.865$, $P < 0.03$), whereas fasting glucose production, measured with D-[6,6-$^2H_2$]glucose, decreased by ~10% ($P < 0.04$). In conclusion, these results indicate that IMCLs relate to insulin resistance in type 2 diabetic patients at baseline and that insulin-mediated near-normoglycemia for ~3 days reduces fasting glucose production but stimulates lipid accumulation in liver and muscle without affecting insulin sensitivity. *Diabetes* 51:3025–3032, 2002

From the Division of Endocrinology and Metabolism, Department of Internal Medicine III, University of Vienna, Vienna, Austria.

Address correspondence and reprint requests to Michael Roden, Division of Endocrinology and Metabolism, Department of Internal Medicine III, University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria. E-mail: michael.roden@akh-wien.ac.at.

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C-D1, study day 1 for control subjects; EGP, endogenous glucose production; FFA, free fatty acid; IHCL, intrahepatocellular lipid; IMCL, intramyocellular lipid; IMCL-S, IMCLs in soleus muscle; IMCL-TA, IMCLs in tibialis anterior muscle; MCR, metabolic clearance rate; MPE, mole percent excess; NMR, nuclear magnetic resonance; P-D1, study day 1 for type 1 diabetic patients; P-D2, study day 2 for type 2 diabetic patients; P-D3, study day 3 for type 2 diabetic patients; P-D6, study day 6 for type 2 diabetic patients.

Insulin resistance is commonly defined by reduced insulin sensitivity of peripheral tissues, such as skeletal muscle and fat, and represents the major abnormality of the metabolic syndrome and type 2 diabetes (1). Likewise, impaired suppression by insulin of endogenous glucose production (EGP) in these disorders is considered to indicate hepatic insulin resistance (2), which correlates with plasma free fatty acids (FFAs) (3) and intrahepatocellular lipids (IHCLs) (4–6).

Increased plasma FFAs also reflect skeletal muscle insulin resistance (7,8) and induce a rise in intramyocellular lipid (IMCL) content at high but not low plasma insulin concentrations (9–11). In some muscles, IMCLs correlate negatively with whole-body glucose disposal in both insulin-sensitive and -resistant nondiabetic subjects (12–14). Recently, evidence was provided that subjects with increased IMCLs (15) exhibit impaired insulin signal transduction in skeletal muscle (7,8,11,14,16). However, it is not clear whether muscle lipid accumulation primarily induces insulin resistance by defects in insulin signaling or is simply a secondary phenomenon of impaired insulin sensitivity due to reduced lipid oxidation (17).

In insulin-resistant states, including type 2 diabetes, abnormalities in insulin signaling (18) coexist with increased plasma insulin concentrations (19), but with progression of the disease, impaired insulin secretion fails to compensate for the decrease in insulin sensitivity (20). The resulting rise in plasma glucose can be normalized by insulin treatment at the expense of peripheral hyperinsulinemia (21). Thereby, insulin not only stimulates lipogenesis, but could also increase IMCLs and IHCLs and alter whole-body insulin sensitivity (21–24).

This study was therefore designed to examine the relationship between whole-body insulin sensitivity and EGP with IMCLs and IHCLs before as well as after short-term (12-h) and prolonged (67-h) insulin-mediated near-normoglycemia in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study participants. Eight type 2 diabetic patients with known duration of disease ≤10 years were recruited. They were hyperglycemic, and most of them were overweight and dyslipidemic (Table 1). None had been treated with insulin before or presented with type 1 diabetes–related antibodies (25) or any liver or kidney diseases. Type 2 diabetic patients with hypertension, cardiovascular diseases, or diabetic late complications were excluded from the study. Female patients were postmenopausal. Treatment with diuretics was

TABLE 1
Baseline clinical characteristics as well as fasting plasma/serum laboratory parameters of eight type 2 diabetic patients

	F.K.	H.H.	M.A.	H.G.	A.M.	I.H.	M.L.	H.M.	Means ± SE
Sex	M	M	F	M	M	F	M	M	
Age (years)	59	67	56	56	66	59	68	57	60 ± 2
Known duration of diabetes (years)	4	5	3	1.5	1	5	8	10	4.7 ± 1.1
Weight (kg)	87.0	65.0	68.0	89.0	77.5	96.6	76.3	91.8	81.4 ± 4.0
BMI (kg/m^2)	30.5	22.8	25.3	29.1	28.5	33.8	28.0	29.6	28.4 ± 1.2
HbA_{1c} (%)	8.9	8.8	8.1	8.6	7.5	9.7	8.4	9.1	8.6 ± 0.2
Glucose (mg/dl)	234	395	366	210	144	214	237	278	260 ± 30
Insulin (mU/l)	15	10	9	15	12	7	6	6	10 ± 1
FFA (μmol/l)	140	604	937	890	891	596	716	945	715 ± 54
Triglycerides (mg/dl)	471	335	234	275	245	112	437	152	283 ± 45
Total cholesterol (mg/dl)	231	294	273	231	238	193	263	178	238 ± 14
HDL cholesterol (mg/dl)	39	55	52	47	26	37	43	29	41 ± 4
LDL cholesterol (mg/dl)	150	116	123	101	205	190	183	147	152 ± 13
AST (units/l)	9	10	11	6	7	8	7	6	8 ± 1
ALT (units/l)	13	18	14	13	9	14	9	9	12 ± 1
Creatinine (mg/dl)	0.99	1.13	0.85	0.93	0.90	0.77	1.04	0.95	0.95 ± 0.04

ALT, alanine transaminase; AST, aspartate transaminase.

discontinued for at least 1 week and any other medication, including oral hypoglycemic agents, for at least 3 days before the study.

The control group consisted of healthy nondiabetic subjects (four men and two women, aged 57 ± 2 years, body weight 78 ± 6 kg, BMI 25.7 ± 1.1 kg/m^2; NS vs. type 2 diabetic patients) exhibiting normal fasting and 2-h plasma glucose during a standard 75-g oral glucose challenge. Fasting plasma glucose (99 ± 1 mg/dl), HbA_{1c} (5.5 ± 0.1%), FFAs (556 ± 46 μmol/l), and triglycerides (122 ± 27 mg/dl) were lower (each $P < 0.02$), whereas serum cholesterol (total 202 ± 14 mg/dl, HDL 55 ± 9, LDL 123 ± 12) was similar to type 2 diabetic patients (Table 1). Control subjects also had normal liver and kidney function (serum creatinine 1.1 ± 0.0 mg/dl, aspartate transaminase 10 ± 0 units/l, alanine transaminase 10 ± 1 units/l). All participants gave informed consent to the protocol, which was approved by the institutional ethics board.

Study design. Type 2 diabetic and control subjects were instructed to ingest an isocaloric carbohydrate-rich diet from day −7 onwards. Type 2 diabetic patients were admitted to the hospital at least 1 day before the studies and given an isocaloric diet (25 kcal · day^{-1} · kg^{-1} body wt; 50% carbohydrate, 15% protein, and 35% fat) divided into five meals (7:30 A.M., 11:00 A.M., 12:30 P.M., 4:30 P.M., and 7:00 P.M.) until day 6.

Intracellular lipids, EGP, and whole-body insulin sensitivity were measured after a fast for at least 10 h on study days 1 (P-D1), 3 (P-D3), and 6 (P-D6) for type 2 diabetic patients and on study day 1 for control subjects (C-D1). At 5:50 A.M., two catheters (Vasofix; Braun, Melsungen, Germany) were inserted into one antecubital vein of the left and right arm for blood sampling and infusions, respectively. Starting at 6:00 A.M., a primed-continuous infusion (5 min: 3.6 mg × kg body · wt × fasting glucose [mg/dl]/90 [mg/dl]; 475 min: 0.036 mg/min × kg body · wt) of D-[6,6-$^2H_2$]glucose (98% enriched; Cambridge Isotope Laboratories, Andover, MA) was performed until the end of the clamp to determine EGP (26). At 7:00 A.M., all participants were transported by wheelchair to the MR Unit to measure IHCLs and IMCLs. From 9:00 A.M. to 2:00 P.M., three stepped hyperinsulinemic-euglycemic clamps were performed, with infusion of insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) at rates of 1.0, 2.0, and 4.0 mU · min^{-1} · kg^{-1} for 100 min each. In type 2 diabetic patients, plasma glucose was allowed to decrease to 100 mg/dl and then kept at 102 ± 5, 99 ± 2, and 100 ± 3 mg/dl using variable infusion rates of 20% glucose during clamps 1, 2, and 3 on P-D1, P-D3, and P-D6, respectively (Table 2). In control subjects, plasma glucose was maintained at 98 ± 3 mg/dl during the clamp. All 20% glucose infusions were enriched with D-[6,6-$^2H_2$]glucose to 2.0 ± 0.0% mole percent excess (MPE) (11). On P-D1, P-D3, and P-D6, the first and second meal was omitted to compensate for the amount of infused glucose on these days. In type 2 diabetic patients, mean plasma glucose was maintained at 120 ± 8 mg/dl (range 92–189) for 12 h before clamp 2 (i.e., from study day 2 for type 2 diabetic patients [P-D2] at 9:00 P.M. to P-D3 at 9:00 A.M.) as well as immediately after clamp 2 for 67 h before clamp 3 (i.e., from P-D3 at 2:00 P.M. to P-D6 at 9:00 A.M.), with intravenous insulin infusion (1 unit/ml Actrapid) at variable rates (mean 2.5 ± 0.8 units/h, range 0–17).

In vivo ^{1}H nuclear magnetic resonance spectroscopy. The participants were studied in supine position within a 3.0-T/80-cm nuclear magnetic resonance (NMR) spectrometer (Medspec; Bruker, Ettlingen, Germany) equipped with a whole-body gradient coil (BG-A55: 40 mT m^{-1}, <250 μs). First, the calf muscle of the right leg was positioned in a standard 28-cm quadrature birdcage ^{1}H volume coil for measurement of IMCLs in soleus (IMCL-S) and tibialis anterior muscle (IMCL-TA). Thereafter, a 10-cm circular ^{1}H surface coil was positioned over the livers' lateral aspect for IHCL measurement. The STEAM sequence parameters (echo time = 20 ms, mixing time = 30 ms, repetition time [TR] = 6 s, and number of scans [NS] = 8 and 32) (27) were complemented by CHESS water suppression and applied on the volume of interest placed in soleus and tibialis anterior muscle (1.2 cm) (3) and liver (27 cm) (3). IMCL-S and -TA were quantified from line-broadened and line-fitted processed spectra as the percent relation of the intensity of $(CH_2)_n$ group resonance (1.25 ppm) to the intensity of the water resonance from non–water-suppressed spectra of the same volume of interest (11). IHCL was quantified from processed spectra as the ratio between the sum of intensities of both $(CH_2)_n$ (1.25 ppm) and CH_3 (0.8–0.9 ppm) group resonances and the intensity of the water resonance. ^{1}H NMR spectroscopy was not performed in the first two patients (F.K. and H.H.). For assessment of intraindividual day-to-day variability and variance within one single day of the measurements, three healthy fasted subjects (two men and one women, aged 28 ± 2 years, BMI 22 ± 1 kg/m^2) were examined in the morning of 2 separate days. Each day, three repetitive measurements of liver and both muscles were performed. In absolute terms, the day-to-day variability of IMCL-S, IMCL-TA, and IHCLs was 0.21 ± 0.03, 0.04 ± 0.01, and 0.67 ± 0.28% of the intensity of water resonance, respectively. Mean values between both days were not different ($P > 0.55$). Comparison of repetitive measurements within one single day gave an intraindividual coefficient of variation of 5–10% for both IMCLs and IHCLs.

Plasma metabolites and hormones. Plasma glucose concentrations were measured using the glucose oxidase method (Glucose Analyzer II; Beckman, Fullerton, CA). Plasma immunoreactive insulin and C-peptide were analyzed by commercially available radioimmunoassays (insulin: Pharmacia, Uppsala, Sweden; C-peptide: Cis, Gif-Sur-Yvette, France) and plasma FFA concentrations with a microfluorimetric assay (Wako, Richmond, VA).

MPE of plasma and infusate [$^2H_2$]glucose was measured on a Hewlett-Packard 5890 gas chromatograph equipped with a CP-Sil5 25 m × 0.25 mm × 0.12 μm capillary column (Chrompack, Middelburg, the Netherlands) and interfaced to a Hewlett-Packard 5971A Mass Selective Detector as described (11).

Calculations. Baseline rates of EGP were calculated by dividing the tracer (D-[6,6-$^2H_2$]glucose) infusion rate times tracer enrichment by the tracer enrichment in plasma and subtracting the tracer infusion rate (28). During the clamps, EGP was assessed using the formula: $EGP = GIR_{mean} \times [(enrichment_{inf}/enrichment_{plasma}) - 1]$, where GIR_{mean} is the mean glucose infusion rate during the preceding 35 min, $enrichment_{inf}$ is the MPE of glucose in infusate, and $enrichment_{plasma}$ is the MPE of plasma glucose during steady-state conditions of the clamp.

M values and *M/I* ratios were calculated from the glucose infusion rate during the clamps, corrected for glucose space and urinary glucose loss (29). Glucose metabolic clearance rates (MCRs) during steady-state conditions (i.e., the last 40 min of each clamp step) were calculated by dividing *M* by steady-state plasma glucose (30).

Statistical analyses. All data are given as means ± SE. Intraindividual comparisons within each group were analyzed with the two-tailed paired Student's *t* test. Comparisons between both groups were done with the

FIG. 1. Changes in plasma glucose (mg/dl, upper panel) and rates of insulin infusion (units/h, lower panel) during near-normoglycemic metabolic control for 12 h (P-D2 to P-D3) before clamp 2 (*A*) and 67 h (P-D3 to P-D6) before clamp 3 (*B*) in type 2 diabetic patients (n = 8). Arrows indicate ingestion of meals. Data are given as means ± SE

two-tailed unpaired Student's t test. Comparisons of more than two variables were calculated with ANOVA after post hoc Dunnett t test. Linear correlations are Pearson product moment correlations. Differences were considered statistically significant at $P < 0.05$. All statistical analyses were performed using Microsoft Excel 97 or SPSS version 10.0.7 for Windows (SPSS, Chicago).

RESULTS

Plasma glucose profiles and insulin infusion rates in type 2 diabetic patients

Insulin infusion (12 h). To restore euglycemia after dinner on P-D2, 7.6 ± 0.8 units of insulin were required to lower plasma glucose from 258 ± 25 to 128 ± 13 mg/dl within 2 h (Fig. 1*A*). After 3 h, plasma glucose declined below 120 mg/dl and remained at 113 ± 2 mg/dl until 9:00 A.M. of P-D3. During nighttime, the mean insulin infusion rate for maintenance of euglycemia was 1.7 ± 0.2 units/h.

Insulin infusion (67 h). Prolonged insulin infusion effectively normalized plasma glucose over the course of 67 h (overall mean 118 ± 7 mg/dl). During nighttime (from 11:00 P.M. of P-D3 to 7:00 A.M. of P-D6), plasma glucose remained at 105 ± 2 mg/dl (range 92–120) and rose to 127 ± 7 mg/dl (92–189) during daytime (7:00 A.M. to 11:00 P.M.). Mean insulin infusion rates for 67 h were 2.5 ± 0.8 units/h (range 0–17) (Fig. 1*B*), with individual peak rates of 5–24 mU · min^{-1} · kg^{-1}. Due to meal ingestion during daytime, insulin requirement was almost threefold higher (3.3 ± 0.6 units/h) than during nighttime (1.2 ± 0.1 units/h).

Clamp results

Metabolites and hormones. The fasting plasma glucose of type 2 diabetic patients before clamp 1 was about two times higher than that before clamps 2 and 3 (each $P <$ 0.01), which were not different from that of control subjects (Tables 2 and 3). $P < 0.03$ vs. C-D1; 95%, $P <$ 0.00001 vs. P-D3; 40%, $P < 0.003$ vs. P-D6) but fell during overnight insulin infusion on P-D3 (−48%, $P < 0.00001$ vs. P-D1) and were not different from those of control subjects (C-D1) on P-D3 and P-D6 (Tables 2 and 3). Due to treatment of type 2 diabetic patients with exogenous insulin before clamp 2 ($P < 0.02$ vs. P-D1) and clamp 3

TABLE 2
Data of clamp 1 in diabetic patients (n = 8) and healthy control subjects (n = 6)

	Clamp 1 of diabetic patients (P-D1)			Clamp 1 of control subjects (C-D1)		
	Basal	1.0	2.0	4.0	Basal	1.0	2.0	4.0
Plasma glucose (mg/dl)	192 ± 20	112 ± 7*	97 ± 2*	96 ± 3*	100 ± 3†	103 ± 2	95 ± 1	99 ± 3
Plasma FFA (μmol/l)	715 ± 54	122 ± 17*	36 ± 10*	20 ± 6*	556 ± 46†‡	38 ± 5†‡§‖	16 ± 3§	10 ± 0§
Plasma insulin (mU/l)	10 ± 1	77 ± 3*	181 ± 19*	412 ± 34*	7 ± 2¶#	68 ± 5§	141 ± 18§	386 ± 36§
M (mg glucose · min^{-1} · kg^{-1})	—	2.9 ± 0.2	5.1 ± 0.3	7.3 ± 0.4	—	6.0 ± 0.7†‡#	8.4 ± 0.6†‡#	10.7 ± 0.9†‡#
M/I (mg · min^{-1} · kg^{-1} · mU^{-1} · l × 100)	—	3.9 ± 0.5	3.1 ± 0.4	2.0 ± 0.3	—	9.4 ± 1.8†‡#	7.6 ± 1.7†‡#	2.8 ± 0.4¶‖
Glucose MCR (ml · min^{-1} · kg^{-1})	—	2.4 ± 0.3	5.2 ± 0.4	7.8 ± 0.7	—	6.1 ± 0.7†‡#	8.5 ± 0.6†‡#	10.7 ± 0.8‡¶
EGP (mg · min^{-1} · kg^{-1})	2.0 ± 0.1	0.4 ± 0.5*	0.0 ± 0.3*	−0.3 ± 0.3*	1.7 ± 0.0	−0.7 ± 0.1§	−1.0 ± 0.2¶§	−1.0 ± 0.2¶§

Data are means ± SE. Metabolites and hormones as well as M, M/I, glucose MCR, and EGP during steady-state conditions (60–100 min, 160–200 min, and 260–300 min) before (basal) and during the clamps performed on P-D1 and C-D1, respectively. *$P < 0.05$ vs. basal conditions before clamp 1 (Student's t test). †$P < 0.01$ vs. respective values from clamp 1 in type 2 diabetic patients. ‡$P < 0.01$ vs. respective values from clamp 2 in type 2 diabetic patients (Table 3). §$P < 0.05$ vs. basal conditions of control subjects (Student's t test). ‖$P < 0.05$ vs. respective values from clamp 3 in type 2 diabetic patients. (Table 3). ¶$P < 0.05$ vs. respective values from clamp 2 in type 2 diabetic patients (Table 3). #$P < 0.01$ vs. respective values from clamp 3 in type 2 diabetic patients (Table 3). **$P < 0.05$ vs. respective values from clamp 1 in type 2 diabetic patients.

TABLE 3
Data of clamps 2 and 3 in diabetic patients

Insulin infusion rates (mU · min^{-1} · kg^{-1})	Clamp 2 (P-D3)				Clamp 3 (P-D6)			
	Basal	1.0	2.0	4.0	Basal	1.0	2.0	4.0
Plasma glucose (mg/dl)	112 ± 6*	102 ± 2	95 ± 3	99 ± 1	115 ± 7*	102 ± 2	104 ± 2	97 ± 2
Plasma FFA (μmol/l)	374 ± 23†	98 ± 14‡§	36 ± 10§	18 ± 5§	522 ± 46*‖	104 ± 24§	32 ± 42§	20 ± 4§
Plasma insulin (mU/l)	15 ± 2‡	69 ± 3§	170 ± 14§	437 ± 45§	24 ± 4*	74 ± 12§	153 ± 7§	407 ± 15§
M (mg glucose · min^{-1} · kg^{-1})	—	2.6 ± 0.1	5.3 ± 0.2	7.4 ± 0.2	—	2.4 ± 0.2	4.8 ± 0.2	7.5 ± 0.3
M/I (mg glucose · min^{-1} · kg^{-1} · l × 100)	—	4.0 ± 0.5	3.2 ± 0.3	1.9 ± 0.2	—	3.1 ± 0.3	3.1 ± 0.3	1.8 ± 0.2
Glucose MCR (ml · min^{-1} · kg^{-1})	—	2.6 ± 0.3	5.5 ± 0.3	7.4 ± 0.4	—	2.3 ± 0.4	4.8 ± 0.3	7.7 ± 0.7
EGP (mg · min^{-1} · kg^{-1})	2.0 ± 0.1	−0.2 ± 0.3‡§	−0.2 ± 0.1§	−0.2 ± 0.1§	1.8 ± 0.1*¶	−0.6 ± 0.4‡§	−0.5 ± 0.3§	−0.7 ± 0.4§

Data are means ± SE (n = 8) Metabolites and hormones as well as M, M/I, glucose MCR, and EGP during steady-state conditions (60–100 min, 160–200 min, and 260–300 min) before (basal) and during clamps 2 and 3 performed on P-D3 and P-D6, respectively. *P < 0.01, †P < 0.001, ‡P < 0.05 vs. respective values from clamp 1 (Table 2). §P < 0.05 vs. basal conditions before respective clamp (Student's t test). ‖P < 0.01, ¶P < 0.05 vs. respective values from clamp 2.

FIG. 2. ***A*: Time course of M values (in milligrams glucose per minute per kilogram) during euglycemic-hyperinsulinemic clamps (1.0, 2.0, and 4.0 mU insulin · min^{-1} · kg^{-1}, each for 100 min) in both type 2 diabetic patients (n = 8) on P-D1 (clamp 1, ●), P-D3 (clamp 2, ♦) and P-D6 (clamp 3, ▲) and nondiabetic control subjects (n = 6) on C-D1 (○). *B*: Suppression of EGP at different clamp steps (as indicated) of both type 2 diabetic patients on days 1 (clamp 1, ●), 3 (clamp 2, ♦), and 6 (clamp 3, ▲), and control subjects (□). *C*: Relationship between basal EGP and basal plasma glucose concentration before clamp 1 in both type 2 diabetic patients (●) and control subjects (○). Data are given as means ± SE. ⋆P < 0.02 vs. control subjects (ANOVA, Dunnett post hoc); ¶P < 0.05 vs. P-D1 (Student's t test) .**

(P < 0.02 vs. P-D1, P < 0.04 vs. C-D1), plasma insulin was higher before clamps 2 and 3, but not different during the steady-state period of each clamp. At baseline (P-D1), plasma C-peptide was similar in type 2 diabetic and control subjects (1.9 ± 0.2 vs. 1.6 ± 0.2 μg/l). Insulin treatment reduced basal C-peptide in type 2 diabetic patients (0.9 ± 0.1 μg/l, P < 0.05 vs. P-D1; P < 0.001 vs. C-D1).

Glucose metabolism. In all clamps of type 2 diabetic patients, both M and glucose MCR were decreased by 3.3 ± 0.2 and 3.4 ± 0.1 ml · min^{-1} · kg^{-1}, respectively (each P < 0.02 vs. control subjects) (Fig. 2*A* and Tables 2 and 3). At each step of all three clamps, M, M/I, and glucose MCR were not different in type 2 diabetic patients (Fig. 2*A* and Tables 2 and 3). Control subjects exhibited lower fasting EGP (1.7 ± 0.1 ml · min^{-1} · kg^{-1}) than type 2 diabetic patients before long-term insulin infusion (P-D1 and P-D3: 2.0 ± 0.1 mg · min^{-1} · kg^{-1}, each P < 0.002 vs. C-D1) (Tables 2 and 3). However, after the 67-h insulin infusion in type 2 diabetic patients, EGP was reduced by ~10% compared with that before clamps 1 and 2 (P < 0.04 vs. P-D1 and P-D3) but similar to that of control subjects (Tables 2 and 3). In type 2 diabetic patients, suppression by low-dose insulin (1 mU · min^{-1} · kg^{-1}) of EGP was less pronounced at baseline (P-D1, each P < 0.05 vs. C-D1, P-D3, and P-D6), whereas it was similar to that of control subjects after short- (P-D3) and long-term (P-D6) insulin

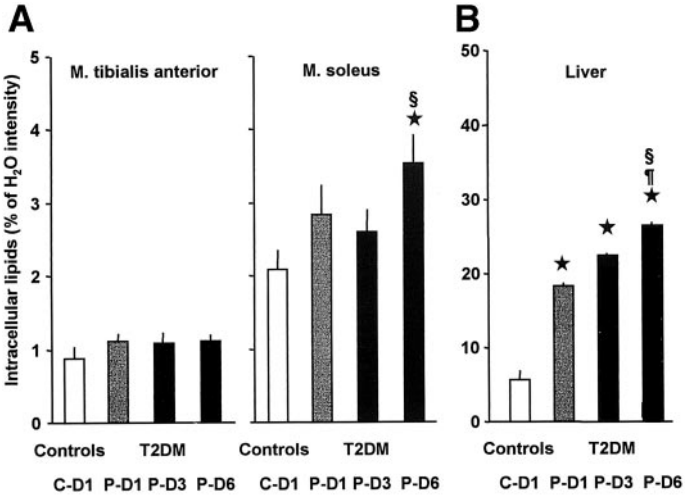

FIG. 3. Intracellular lipids (means ± SE) in tibialis anterior (*A*) and soleus muscle (*B*) and liver. Lipids were measured between 7:00 and 8:45 A.M. in control subjects (n = 6, □) on C-D1 as well as in type 2 diabetic patients (T2DM, n = 6) on P-D1, P-D3, and P-D6 by ^{1}H NMR spectroscopy. ⋆P < 0.03 vs. C-D1; §P < 0.05 vs. P-D3; ¶P < 0.05 vs. P-D1 (ANOVA with Dunnett post hoc test or Student's t test) .

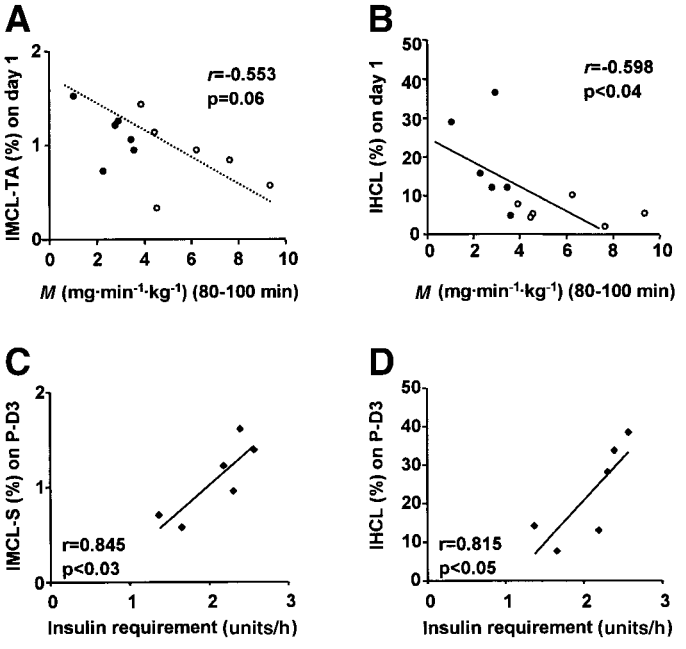

FIG. 4. Correlation of clamp *M* values during the 80- to 100-min interval on C-D1 and P-D1 with IMCL-TA (●, type 2 diabetic patients: r = −0.696, P < 0.001; ○, control subjects: r = −0.441, P = 0.381) (*A*) and IHCLs (●, type 2 diabetic patients: r = −0.517, P = 0.29; ○, control subjects: r = −0.244, P = 0.641) (*B*). Correlation of the amount of insulin (in units per hour) required for 67 h of near-normoglycemic control (from P-D3 to P-D6) with IMCL-S (*C*) and IHCLs (*D*), both measured on P-D3 (i.e., before long-term insulin infusion).

infusion (Fig. 2*B*). In contrast, suppression by high-dose insulin (2 and 4 mU · min^{-1} · kg^{-1}) of EGP was comparable between type 2 diabetic and control subjects (Fig. 2*B*).

Basal EGP positively correlated with fasting plasma glucose in both groups combined (type 2 diabetic and control subjects: r = 0.764, P < 0.0003) and in type 2 diabetic patients alone (P-D1: r = 0.811, P < 0.01) (Fig. 2*C*).

Intracellular lipids. At baseline, IMCL-TA (P-D1: 1.12 ± 0.10% vs. C-D1: 0.87 ± 0.16%; P = 0.24) and IMCL-S (P-D1: 2.83 ± 0.42% vs. C-D1: 2.14 ± 0.25%; P = 0.23) were not significantly different between type 2 diabetic and control subjects (Fig. 3*A*). However, IHCLs were more than threefold higher in type 2 diabetic patients than in control subjects (P < 0.03) (Fig. 3*B*). In type 2 diabetic patients, short-term insulin infusion (12 h) did not affect intracellular lipids, whereas prolonged insulin treatment (67 h) increased both IMCL-S and IHCLs by ~36 and ~18%, respectively (P < 0.05 vs. P-D3) (Fig. 3).

Correlation analyses

P-D1 and C-D1. In type 2 diabetic patients, *M* (80–100 min) negatively correlated with IMCL-TA (r = −0.969, P < 0.001) (Fig. 4*A*) but not with IMCL-S and IHCLs (individual data not shown). In both groups combined (control and type 2 diabetic subjects), the relationship between IMCL-TA and *M* (80–100 min) did not obtain statistical significance (r = −0.553, P = 0.06), which can be explained by one outlier in the control group (Fig. 4*A*). In addition, IHCLs negatively correlated with *M* (80–100 min) in all participants (r = −0.598, P < 0.04) (Fig. 4*B*). Basal plasma insulin concentrations were also inversely related to *M* (80–100 min) in all participants (r = −0.568, P < 0.05) (individual data not shown).

P-D3. Both IMCL-TA and IHCLs on P-D3 (i.e., before the start of the 67-h insulin infusion) directly correlated with the amount of insulin required to maintain normoglycemia during the subsequent 67 h between P-D3 and P-D6 (r = 0.845, P < 0.03 and r = −0.815, P < 0.05, respectively) (Fig. 4*C* and *D*).

P-D6. After prolonged insulin infusion, *M* (80–100 min) was positively associated with IMCL-S (r = 0.982, P < 0.0005) and IHCLs (r = 0.864, P < 0.03) (Fig. 5*A* and *B*) but not with IMCL-TA. Moreover, IMCL-S and IHCLs correlated with each other (r = 0.823, P < 0.04) (individual data not shown). In addition, basal plasma FFAs were inversely related to IMCL-S (r = −0.897, P < 0.01) and *M* (80–100 min) of clamp 3 (r = −0.826, P < 0.04) (Fig. 5*C* and *D*).

DISCUSSION

The present study found that in non–insulin-treated type 2 diabetes, lipid content in tibialis anterior muscle negatively correlates with insulin sensitivity at baseline, whereas after prolonged insulin infusion, lipid content in both soleus muscle and liver is increased and relates positively to insulin sensitivity.

Comparison of type 2 diabetic and control subjects at baseline. Before initiation of the insulin infusion and near-normoglycemia on day 1, type 2 diabetic patients presented with lower *M* and glucose MCRs and higher fasting EGP than the healthy control subjects, indicating insulin resistance in skeletal muscle and liver, which is typical for type 2 diabetes (23). Although the broad range of body mass might suggest different phenotypes of type 2 diabetes, our type 2 diabetic patients exhibited similar degrees of insulin resistance and fasting EGP and uniformly tested negative for type 1 diabetes–related antibodies (25). In type 2 diabetic patients, insulin sensitivity correlated negatively with IMCLs in tibialis anterior but not in soleus muscle. These results are partly in line with studies in young insulin-resistant but nondiabetic offspring of type 2 diabetic patients, in whom whole-body insulin sensitivity was linked to increased IMCLs in both muscles

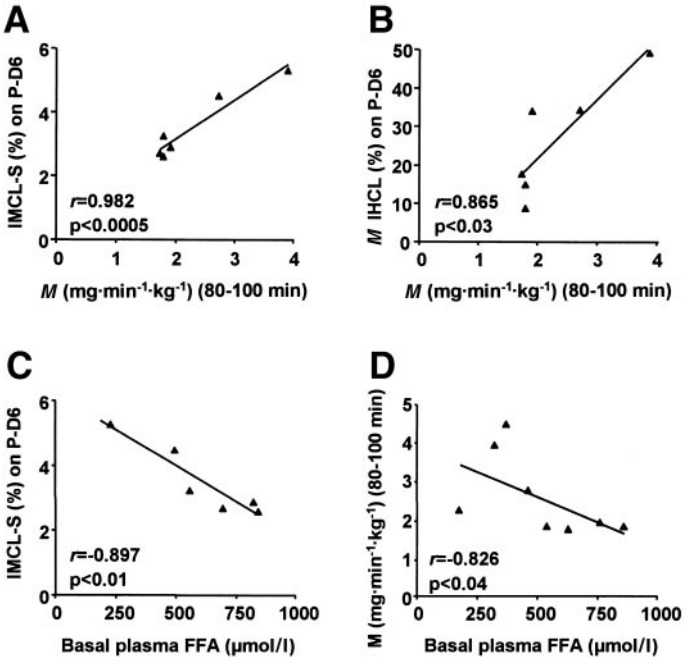

FIG. 5. Correlation of clamp *M* values (in milligrams glucose per minute per kilogram, 80- to 100-min interval on P-D6) after a 67-h insulin infusion with IMCL-S (*A*) and IHCLs (*B*) (type 2 diabetic patients, $n = 6$). Relationships of basal plasma FFAs with IMCL-S (*C*) and *M* values (*D*) (80- to 100-min interval of clamp 3) on P-D6 (type 2 diabetic patients, $n = 6$).

(15) or in soleus muscle only (13). Despite the lower *M* values, IMCLs in type 2 diabetic patients and control subjects were comparable, although type 2 diabetic patients tended to exhibit higher IMCLs than control subjects. Of note, our healthy control subjects were neither young nor lean; therefore, the lack of any difference might be partly explained by the higher age and body fat mass of both groups. On the other hand, IHCLs were clearly higher in type 2 diabetic patients than in control subjects and correlated negatively with whole-body insulin sensitivity. These data suggest that IHCL concentration is more tightly related to insulin resistance. In addition, our findings confirm the positive correlation between fasting EGP with fasting plasma glucose concentration (31,32).

Insulin infusion studies. Before clamp 3 (P-D6) after long-term insulin infusion, basal EGP decreased (−10% vs. baseline conditions) to values similar to those of control subjects. This effect was preceded by a somewhat improved insulin-induced suppression of EGP during the first step of clamp 2 after short-term insulin infusion. In parallel, baseline plasma FFAs on P-D6 were also decreased to the range of nondiabetic subjects. Thus, our results support recent observations that overnight normalization of plasma glucose by insulin reduces EGP and plasma FFAs but not the degree of whole-body insulin resistance in type 2 diabetic patients (33). In addition, impaired suppression by low-dose insulin of EGP of only ~50% is in line with recent results obtained under comparable clamp conditions that were ascribed to reduced insulin-dependent inhibition of gluconeogenesis (34). Of note, FFAs inhibit insulin signaling in hepatocytes at both receptor and postreceptor steps (35) and can stimulate gluconeogenesis (36–38). It is therefore conceivable that long-term normalization of plasma FFAs is responsible for the reduction in basal EGP, whereas short-term decrease of FFAs predominantly improves insulin-mediated suppression of EGP (39).

During short-term and prolonged insulin infusion, plasma glucose profiles were similar to those of control subjects and type 1 diabetic patients on intensified insulin therapy (40). However, mean insulin infusion rates (2.5 units/h) more than twofold above the insulin requirement of healthy subjects (41) were necessary to maintain plasma glucose within the physiological range for 67 h. This also reflects a considerable degree of insulin resistance, which did not change in the course of the study. Similarly, euglycemia for 10 days resulted in increased plasma insulin response to an oral glucose challenge but did not improve whole-body insulin sensitivity during 1 $mU \cdot kg^{-1} \cdot min^{-1}$ clamps in slightly obese type 2 diabetic patients (22). From this it appears that improvement of insulin sensitivity requires normoglycemia for at least 2 weeks in both type 2 (21,23,24) and type 1 diabetic patients (40,42).

After prolonged insulin infusion (P-D6), IHCLs and IMCL-S were increased and, surprisingly, correlated positively with insulin sensitivity, which is in contrast to the baseline conditions (P-D1). The rise in intracellular lipids could have resulted from *1*) insulin-mediated inhibition of lipolysis and lipid oxidation, *2*) insulin-mediated stimulation of intracellular triglyceride synthesis, and/or *3*) increased uptake of circulating FFAs. The 67 h of hyperinsulinemia not only normalized plasma glucose but also reduced plasma FFA concentrations, indicating insulin-stimulated inhibition of lipolysis. The increased availability of lipids such as IMCLs could then induce insulin resistance by increasing cytosolic long-chain fatty-acyl CoA. Alternatively, insulin resistance has been suggested to be mainly responsible for the inflexibility to regulate fat oxidation and thereby lead to increased IMCLs (43). In the present study, cellular triglyceride contents increased during prolonged insulin infusion despite the unchanged degree of insulin resistance. Finally, in non–insulin-treated type 2 diabetic patients, both reduction of intramuscular triglyceride synthesis and uptake of FFAs have been linked to decreased content of fatty acid–binding protein (44,45). Prolonged exposure to insulin in vitro leads to increased uptake and esterification of FFAs, whereas triglyceride hydrolysis and FFA oxidation are reduced in rodent soleus muscle (46). Regardless of the mechanism, because whole-body insulin sensitivity remained unchanged, it appears that insulin per se increases IMCLs, whereas accumulation of IMCLs does not necessarily deteriorate insulin sensitivity in skeletal muscle. Taken together, these data support the hypothesis that an increase in IMCLs does not induce insulin resistance but could be a secondary phenomenon resulting from the degree of hyperinsulinemia (15).

Interestingly, intracellular lipid content in tibialis anterior remained unchanged on P-D6 but was increased in soleus muscle. The latter could result from the higher insulin sensitivity and capacity for FFA uptake of soleus muscle, which predominantly consists of type I fibers (13), compared with tibialis anterior muscle, which contains more type IIB fibers (47,48).

Similar to IMCL-S, IHCL was also increased after long-

term insulin infusion. Our findings are in line with studies in hyperinsulinemic rodents, in which the rise in hepatic lipid content was explained by increased activity of lipogenic enzymes, such as acetyl-CoA carboxylase and fatty acid synthase (49). Interestingly, liver fat content has recently been shown to correlate directly with the individual insulin requirements of type 2 diabetic patients (5). Our findings extend this observation by demonstrating that the concentration of intracellular lipids in not only liver but also in tibialis anterior muscle is closely associated with the individual amount of insulin required to achieve normoglycemia in type 2 diabetic patients.

In conclusion, the obtained data demonstrate that *1*) the negative relationship between IMCL content in tibialis anterior muscle and whole-body insulin sensitivity also holds true for type 2 diabetic patients under non–insulin-stimulated conditions and that *2*) insulin treatment for 3 days is sufficient to decrease fasting EGP and plasma FFA concentrations, whereas *3*) insulin induces accumulation of lipids in liver and skeletal muscle without affecting whole-body insulin sensitivity after near-normoglycemia for 67 h.

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