In vivo insulin action and muscle glycogen synthase activity in Type 2 (non-insulin-dependent) diabetes mellitus: effects of diet treatment

J. F. Bak¹, N. Møller², O. Schmitz², A. Saaek¹ and O. Pedersen³

¹ Medical Endocrinological Department C, Aarhus Amtssygehus, ² Second University Clinic of Internal Medicine, Aarhus Kommunehospital, Aarhus, and ³ Steno Diabetes Center, Copenhagen, Denmark

Summary. Insulin resistant glucose metabolism is a key element in the pathogenesis of Type 2 (non-insulin-dependent) diabetes mellitus. Insulin resistance may be of both primary (genetic) and secondary (metabolic) origin. Before and after diet-induced improvement of glycaemic control seven obese patients with newly-diagnosed Type 2 diabetes were studied with the euglycaemic clamp technique in combination with indirect calorimetry and forearm glucose balance. Muscle biopsies were obtained in the basal state and again after 3 h of hyperinsulinaemia (200 mU/l) for studies of insulin receptor and glycogen synthase activities. Similar studies were performed in seven matched control subjects. Insulin-stimulated glucose utilization improved from 110 ± 11 to $183 \pm 23 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ ($p < 0.03$); control subjects: $219 \pm 23 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ ($p = \text{NS}$, vs post-diet Type 2 diabetes). Non-oxidative glucose disposal increased from 74 ± 17 to $138 \pm 19 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ ($p < 0.03$), control subjects: $159 \pm 22 \text{ mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ ($p = \text{NS}$, vs post-diet Type 2 diabetic patients). Forearm blood glucose uptake during hyperinsulinaemia increased from 1.58 ± 0.54 to $3.35 \pm 0.23 \mu\text{mol} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$ ($p < 0.05$), control subjects: $2.99 \pm 0.86 \mu\text{mol} \cdot \text{l}^{-1} \cdot \text{min}^{-1}$

($p = \text{NS}$, vs post-diet Type 2 diabetes). After diet therapy the increase in insulin sensitivity correlated with reductions in fasting plasma glucose levels ($r = 0.97$, $p < 0.001$), reductions in serum fructosamine ($r = 0.77$, $p < 0.05$), and weight loss ($r = 0.78$, $p < 0.05$). Values of muscle glycogen synthase sensitivity to glucose 6-phosphate ($A_{0.5}$ for glucose 6-phosphate) were similar in the basal state. However, insulin stimulation of glycogen synthase was more pronounced after diet treatment ($A_{0.5}$: 0.43 ± 0.06 (before) vs $0.30 \pm 0.04 \text{ mmol/l}$ (after); $p < 0.03$; control subjects: $0.22 \pm 0.03 \text{ mmol/l}$). Muscle insulin receptor binding and kinase activity were similar before and after diet treatment and comparable to values in the control group. The data suggest that impaired insulin stimulation of in vivo glucose turn-over and muscle glycogen synthase activity tend to be restored during successful diet treatment of patients with Type 2 diabetes.

Key words: Insulin resistance, Type 2 (non-insulin-dependent) diabetes mellitus, hyperinsulinaemic clamp, indirect calorimetry, forearm glucose uptake, muscle, glycogen synthase, insulin receptor kinase.

Insulin resistant glucose metabolism plays a pathogenic role in the development of Type 2 (non-insulin-dependent) diabetes mellitus. However, insulin resistance is not a sufficient pathogenic factor since a concomitant reduction (acquired or inherited) in the secretory capacity of the pancreatic Beta cells is also pivotal for the development of diabetes [1].

Insulin resistance of glucose metabolism may have both primary (genetic) and secondary (metabolic) components, the latter being reversible. Thus, reduction of insulin resistance in Type 2 diabetes may be a rational therapeutic intervention to force the malady back into its subclinical state. In Type 2 diabetic patients the insulin resistance often improves during treatment with low-calorie diets [2, 3], whereas overfeeding of lean non-diabetic subjects may have opposite effects [4]. This occurrence may

be attributable both to changes of energy intake per se and to changes in body weight or body composition.

In fat cells from patients with Type 2 diabetes dietary control of hyperglycaemia has been shown to improve glucose metabolism and insulin receptor kinase activity [3, 5]. However, skeletal muscle plays a dominant role in insulin-stimulated glucose disposal [6]. Glycogen synthesis in muscle accounts for a major portion of glucose disposal during hyperinsulinaemia [7]. Moreover, insulin resistance in non-diabetic subjects has been associated with reduced insulin effects on muscle glycogen synthase (GS) [8].

We have previously shown in newly-diagnosed Type 2 diabetic patients that treatment with sulfonylurea was associated with improved insulin sensitivity of glucose metabolism in peripheral tissue and a concomitant potentiated

Table 1. Characteristics of Type 2 (non-insulin-dependent) diabetic patients and control subjects

	Control subjects	Type 2 diabetic patients:	
		Before diet	After diet
n (female/male)	2/5	2/5	—
Age (years)	49 ± 1	51 ± 5	—
Body weight (kg)	78.1 ± 5.2	97.7 ± 4.6	91.5 ± 4.8
Body mass index (kg · m ⁻²)	27.3 ± 1.0	31.3 ± 1.4 ^a	29.3 ± 1.2
s-Fructosamine (μmol/l)	243 ± 7	441 ± 43 ^a	301 ± 18 ^b

^a $p < 0.01$ vs control subjects; ^b $p < 0.01$ vs before diet

insulin activation of muscle GS [9]. The present study in newly-diagnosed Type 2 diabetic patients was carried out to study in vivo glucose metabolism and muscle enzymes with key functions in insulin-controlled glucose metabolism of skeletal muscle, i.e. GS activities, GS conversion rates (i.e. the rate of activation of endogenous GS by endogenous phosphatases), and insulin receptor binding and receptor kinase activity before and after improvement of glycaemic control with hypoenergetic dieting. Data from matched non-diabetic control subjects were included for comparison.

Subjects and methods

Materials

Monoiodinated Tyr-A₁₄-[¹²⁵I]-insulin and native porcine insulin were generously donated by Novo-Nordisk Research Institute (Copenhagen, Denmark). Somatostatin was from Ferring AB (Malmö, Sweden). Uridine diphosphate-[U-¹⁴C]-glucose, adenosine 5'-[γ-³²P]-triphosphate, and [3-³H]-glucose were from New England Nuclear (Boston, Mass., USA). Sepharose-coupled wheat germ agglutinin (WGA) was from Pharmacia (Uppsala, Sweden). All other reagents and chemicals were from Sigma (St. Louis, Mo., USA).

Study design

Nine patients with newly-diagnosed Type 2 diabetes [10] entered the study and were examined at presentation. Patients who responded adequately to 6 weeks of diet treatment, i.e. with a fall in fasting plasma glucose level of more than 4 mmol/l or a post-diet fasting plasma glucose level of less than 7.5 mmol/l were re-examined under conditions similar to the pre-diet examination. Patients who failed to respond sufficiently to diet treatment were excluded from the study and treatment with oral antihyperglycaemic agents was started. For comparison seven healthy non-diabetic subjects with no family history of diabetes or essential hypertension underwent similar examinations. The control subjects were matched for age, whereas their mean body mass index was slightly lower than the mean post-diet body mass index in the diabetic group (Table 1). The study protocol was approved by the ethical committee of the county of Aarhus. Informed consent was obtained from all study participants according to the second Declaration of Helsinki.

Diet composition

Information on diet composition was obtained by a personal interview using a diet history method. All interviews were conducted by a trained dietician applying a structured interview form. Breakfast, lunch, and snacks were recorded on a weekly basis and dinner on a

bi-weekly basis during the periods of intervention. Food models and photographs were used for estimating portion sizes [11]. Nutritional intake was calculated using a computer program based on Danish food composition tables. At the time of diagnosis information on habitual diets was obtained. After the initial examinations patients were prescribed an individual diabetes diet with an energy content of 5000–6000 kJ per day. Follow-up interviews and recordings were performed at the time of the second examination.

Examinations

Each examination lasted 2 days. On day 1 the study participants were examined with a 75 g oral glucose tolerance test (OGTT). On day 2 a euglycaemic clamp study was performed. The clamp experiments commenced at 08.00 hours. A teflon catheter was inserted into an antecubital vein for the administration of infusates (insulin, glucose, and 3-³H-glucose). In the contralateral extremity two catheters were inserted as follows: one catheter was introduced into a wrist vein of the hand which was kept in a heated box for sampling of arterialized blood and another catheter was inserted retrogradely into a deep antecubital vein draining forearm muscles for sampling of deep venous blood. Forearm blood flow per liter total forearm volume was determined with venous occlusion plethysmography using a strain gauge technique [12]. Forearm glucose uptake was calculated by multiplying total forearm blood flow and forearm arteriovenous glucose differences.

The experiments consisted of a 'basal period' of 150 min duration. Subsequently a continuous insulin infusion of 2.0 mU · kg⁻¹ · min⁻¹ was given during the next 180 min. Throughout the clamp 3-³H-glucose was infused continuously (0.30 μCi/min) after a priming dose of FPG/5 × 30 μCi (maximum 60 μCi) where FPG is the fasting plasma glucose level in mmol/l. Batches of [3-³H]-glucose contained no radiochemical contaminants as assessed by HPLC. Plasma glucose was determined by a glucose analyser (Beckman Instruments, Palo Alto, Calif. USA) at 10-min intervals during the clamp study and euglycaemia (5.0 mmol/l) was maintained by a variable glucose infusion. Rates of glucose disappearance were estimated according to the non-steady-state equations of DeBodo et al. [13] from blood sampling every 10 min during the final 30 min of each period.

Indirect calorimetry

Indirect calorimetric measurements with a ventilated hood system (Deltatrac Metabolic Monitor; Datex, Helsinki, Finland) were performed for 20 min at the end of the basal period and again at the end of the hyperinsulinaemic period. In calculating non-protein gaseous exchange, the exchange attributable to protein oxidation was subtracted from total gaseous exchange under the assumption that for each g nitrogen excreted in urine 5.95 litres of O₂ were consumed and 4.968 litres of CO₂ were produced [14]. To determine total nitrogen excretion it was assumed that 90% of the nitrogen appeared in urea [14]. The proportion of non-protein energy derived from fat and carbohydrate oxidation was calculated from the non-protein respiratory quotient as described by Elia et al. [14].

Muscle biopsies

Using a Bergström biopsy needle muscle biopsies were obtained from the vastus lateralis muscle 20 min before termination of the basal period and again 20 min before termination of the hyperinsulinaemic period. Under local anaesthesia (1% lidocaine) a small incision was made through the skin and muscle sheath 15–20 cm above the knee corresponding to the vastus lateralis muscle. The biopsy needle was inserted and a total amount of 200–250 mg muscle was aspirated. The biopsies were cleaned of blood and fat droplets (within 15 s) and immediately deep frozen in liquid ni-

Table 2. Composition of diet

	Before instruction	After instruction	<i>p</i> value
Total energy intake (kJ)	11642 ± 1724	6811 ± 746	< 0.03
Composition (% of energy)			
Fat	38 ± 3	26 ± 1	< 0.03
Protein	14 ± 1	21 ± 1	< 0.03
Carbohydrates	39 ± 3	47 ± 2	< 0.03
Alcohol	10 ± 4	6 ± 3	NS

Table 3. Plasma glucose, insulin, and forearm glucose uptake during clamp experiment

	Control subjects	Type 2 diabetic patients	
		Before diet	After diet
<i>Plasma glucose (mmol/l)</i>			
Basal	5.4 ± 0.1	12.1 ± 1.7 ^a	7.5 ± 0.7 ^b
Hyperinsulinaemia	5.0 ± 0.1	5.2 ± 0.1	5.0 ± 0.1
<i>Plasma insulin (mU/l)</i>			
Basal	12 ± 1	24 ± 6	16 ± 4 ^b
Hyperinsulinaemia	231 ± 16	202 ± 16	194 ± 18
<i>Forearm blood flow (ml · l⁻¹ · min⁻¹)</i>			
Basal	28 ± 4	29 ± 2	28 ± 3
Hyperinsulinaemia	29 ± 3	30 ± 3	29 ± 2
<i>Forearm arteriovenous difference of plasma glucose (mmol/l)</i>			
Basal	0.16 ± 0.04	0.34 ± 0.07 ^a	0.16 ± 0.05 ^b
Hyperinsulinaemia	0.96 ± 0.23	0.55 ± 0.21 ^a	1.19 ± 0.13 ^b
<i>Forearm glucose uptake (μmol · l⁻¹ · min⁻¹)</i>			
Basal	4.2 ± 1.5	10.1 ± 2.4 ^a	5.2 ± 1.9 ^b
Hyperinsulinaemia	29.9 ± 8.6	15.8 ± 5.4	33.5 ± 2.3 ^b

^a *p* < 0.05 vs control subjects; ^b *p* < 0.05 vs before diet

trogen. Muscle samples were stored at -80 °C until the biochemical analyses were performed.

Muscle enzymes

All four biopsies from the same subject were examined on the same day to avoid any influence of day-to-day variations in the assays. Muscle biopsies were homogenized on ice [15] and solubilization of the insulin receptors and subsequent partial purification on WGA sepharose columns were performed in the presence of 1 mmol/l vanadate to inhibit tyrosine phosphatase activity, otherwise as previously described [16]. Insulin binding to WGA-purified receptors was measured with polyethyleneglycol precipitation after incubation for 20 h at 15 °C [16]. The number of insulin binding sites in the eluate was calculated from Scatchard analysis of binding data using the LIGAND computer program of Munson and Rodbard [17]. It was assumed that the curvilinearity of the plots was attributable to a non-specific insulin binding component which was handled as a computer-fitted parameter [16]. Insulin binding data were expressed per 100 mg muscle. The kinase activities of the WGA-purified insulin receptors were measured at room temperature (21 °C) in the presence of 1 mmol/l vanadate after incubation of the receptors with 0, 0.1, 1, 10, and 100 nmol/l insulin for 60 min and with the artificial substrate poly(Glu-Tyr(4:1)) for another 30 min. Phosphorylation with [γ -³²P]-ATP was performed for 60 min at 21 °C [16]. Insulin receptor

kinase activity was adjusted to the number of insulin binding sites in the eluate.

GS activity was measured as described earlier [15] using (final concentrations) 0.13 mmol/l uridine-diphosphate-[U-¹⁴C]-glucose, 0.67% (weight/volume) glycogen, and variable concentrations (0, 0.02, 0.07, 0.20, 0.67, 1.33, 3.33, 6.67 mmol/l) of glucose 6-phosphate (G6P). In the text one unit of GS activity equals the incorporation of one nmol of uridine-diphosphate-glucose into glycogen per min. Total activity of the GS activity is defined as the activity in the presence of a saturating concentration (6.67 mmol/l) of G6P. Fractional velocities were calculated for each G6P concentration as the percentage of total activity [9]. The concentration of G6P giving half-maximal activity of glycogen synthesis ($A_{0.5}$ for G6P) was calculated using a Hill-plot [18]. In ten segments from the same muscle biopsy the inter-assay coefficients of variation were 0.12 for $A_{0.5}$ and 0.13 for total GS activity.

To assess endogenous phosphatase activity the rate of activation of the endogenous GS, i.e. the rate of conversion of the endogenous GS from the G6P-dependent form to the G6P-independent form was measured as previously described [19] under conditions where the phosphatase inhibitor fluoride was left out from the buffers. Briefly, the muscle biopsy was homogenized in an ice-cooled buffer (230 μl buffer/10 mg muscle) containing 50 mmol/l Tris(hydroxymethyl)aminomethane-HCl (Tris-HCl), 10 mmol/l ethylenediaminetetraacetic acid (EDTA), and 50 mmol/l mercaptoethanol, pH 7.8. The homogenate was centrifuged at 6000 *g* for 20 min at 4 °C. From the supernatant 100 μl was diluted with 75 μl buffer and then incubated at 30 °C. The activation reaction was terminated at 0, 5, 10, 15 and 30 min by diluting 26 μl of the mixture in 975 μl of an ice-cold buffer containing 50 mmol/l Tris-HCl, 20 mmol/l EDTA, and 130 mmol/l NaF, pH 7.8. GS activity was measured in the absence and in the presence of 6.7 mmol/l G6P as described above. The rate of conversion of GS was expressed as units of G6P independent form (I-form) of GS produced per min per mg protein. Protein in enzyme extracts was measured according to Bradford et al. [20].

Chemical quantities

Plasma glucose was analysed with a glucose oxidase method (glucose analyser, Beckman Instruments). Plasma insulin and C-peptide concentrations were measured radioimmunologically (radioimmunoassay kits, Novo-Nordisk). Plasma non-esterified fatty acids (NEFA) were determined colorimetrically with a commercial kit (Wako Chemicals, Osaka, Japan). Fructosamine in serum was determined photometrically [21].

Statistical analysis

Data are expressed as means ± SEM. Differences within groups before and after diet treatment were tested for statistical significance with the Wilcoxon matched-pairs test. Differences between groups were tested with the Mann-Whitney test. Correlations were analysed with linear regression statistics. *p* values less than 0.05 were considered statistically significant.

Results

All seven patients who improved in glycaemic control during diet therapy were re-studied approximately 6 weeks after the primary study. The change in diet composition before and after diet instructions is given in Table 2. Improvements of plasma glucose levels during OGTT and of plasma fructosamine concentrations are shown in Figure 1 and Table 1, respectively. Plasma fruc-

Fig. 1. Plasma glucose, serum insulin, serum C-peptide, and plasma non-esterified fatty acids (NEFA) during oral glucose tolerance tests in seven newly-diagnosed obese Type 2 diabetic patients before (○) and after (●) dietary control of hyperglycaemia. Mean values and SEM are shown

tosamine rather than HbA_{1c} was employed to estimate average glycaemic control during previous weeks due to the short duration of the study. Plasma insulin levels in the basal (post-absorptive) state were slightly lower after diet (Table 3, $p = 0.05$). Despite pronounced improvement of glycaemic control, excursions of mean plasma insulin concentrations and mean plasma C-peptide concentrations during OGTT were similar before and after diet intervention (Fig. 1). Also, mean plasma NEFA levels in the basal

state and their decline during OGTT were comparable after diet-induced amelioration of hyperglycaemia (Fig. 1).

During the hyperinsulinaemic step of the clamp experiment plasma insulin levels in diabetic patients were similar before and after diet intervention and also comparable to plasma insulin levels in control subjects (Table 3). Before diet treatment insulin-mediated glucose metabolism and non-oxidative glucose metabolism were significantly ($p < 0.05$) reduced compared to control subjects (Fig. 2). After diet therapy insulin-mediated glucose disposal increased significantly in Type 2 diabetic patients and the major portion of this change was accounted for by an increase in non-oxidative glucose metabolism to levels not significantly different from those in control subjects (Fig. 2). Diet therapy had no significant effect on insulin-stimulated oxidative glucose metabolism (36 ± 11 vs 46 ± 5 $\text{mg} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$, $p = \text{NS}$). The basal state forearm glucose uptake rates decreased after diet therapy of diabetic patients to levels comparable to those in control subjects (Table 3). During hyperinsulinaemia forearm glucose uptake became normal after diet treatment (Table 3). The post-diet increase in net glucose turnover during hyperinsulinaemia correlated significantly with both the fall in fasting plasma glucose level, the fall in plasma fructosamine level, and with weight loss (Fig. 3). In contrast, there was no correlation between the change in total caloric intake and the change in net glucose turnover (Fig. 3).

In Type 2 diabetic patients the recovery of insulin receptors (i.e. maximal insulin binding as calculated from scatchard plots, Fig. 4) was 91 ± 8 fmol/100 mg muscle before diet vs 86 ± 21 fmol/100 mg muscle after diet ($p = \text{NS}$). Maximal insulin binding tended to be higher in control subjects (109 ± 7 fmol receptors/100 mg muscle; Fig. 4), but the difference did not reach statistical significance. Basal and insulin-stimulated receptor kinase activities were unaffected by diet-induced improvement of glycaemic control (Fig. 4) and the insulin receptor kinase activities were not significantly different from values in the control group (Fig. 4). In the post-clamp biopsies insulin receptor binding and kinase activities were similar (data not shown).

Total GS activity (measured with saturating concentrations of G6P and physiological concentrations of uridine-diphosphate glucose) was unaltered after diet treatment and was comparable to values obtained in the control group (Fig. 5). In all subjects total GS activity remained stable during the clamp experiment (Fig. 5). In the basal state the half-maximal activation constant for G6P ($A_{0.5}$) was comparable in all groups and insulin stimulation caused a significant ($p < 0.03$) reduction in $A_{0.5}$ in all groups (Fig. 5). However, during hyperinsulinaemia the lowering of $A_{0.5}$ was more pronounced after diet therapy of Type 2 diabetic patients (0.43 ± 0.06 mmol/l before diet treatment vs 0.30 ± 0.04 mmol/l after diet treatment; $p < 0.03$; control subjects: 0.22 ± 0.03 mmol/l). Thus, whereas GS activation by insulin was significantly ($p < 0.03$) reduced in untreated Type 2 diabetic patients as compared to control subjects, this difference became non-significant after dietary treatment (Fig. 5). There was a

Fig. 2. Insulin-mediated glucose disposal during the hyperinsulinaemic step of the clamp in seven control subjects and seven newly-diagnosed obese Type 2 diabetic patients before and after diet-induced amelioration of glycaemic control. The contribution from non-oxidative glucose metabolism is shown as hatched bars. Mean values and SEM are shown. * $p < 0.03$, ** $p < 0.05$

large variation in the rate of conversion of GS to more active forms (through interactions with phosphatases) and no significant difference was demonstrated in mean values of GS conversion rate between Type 2 diabetic patients and healthy control subjects (Fig. 5). Diet-induced normalization of glycaemic control was not accompanied by significant changes in GS conversion rate (Fig. 5).

Discussion

Prescription of a low-calorie diet is a rational first-choice treatment for Type 2 diabetes, since it may enhance insulin sensitivity. As previously reported [2] improvement of OGTT during diet treatment was associated with a slight reduction in fasting serum insulin levels whereas serum insulin excursions during OGTT were comparable to pre-diet values. Indeed, mean plasma insulin levels during OGTT increased after diet in some subjects whereas it decreased in others. According to the so-called 'Starling curve for insulin secretion' insulin secretion is stimulated with increasing glycaemia until reaching a 'turning point'. Thereafter it is inversely associated with increased hyperglycaemia [22]. In the present study the insulin secretion of some patients may have been on the upward slope of this curve and in others it may have been on the downward slope. This would explain the inconsistency of changes in insulinaemia. Plasma NEFA levels during OGTT were not significantly changed after diet treatment and consequently not likely to be implicated in the amelioration of insulin resistance in this study.

In Type 2 diabetic patients both oxidative and non-oxidative glucose metabolism are impaired during hyperinsulinaemia [23, 24]. However, non-oxidative glucose metabolism is quantitatively dominant under hyperinsulinaemic clamp conditions [24]. A reduced insulin resistance to glucose metabolism after diet treatment is well known from previous studies [2, 3]. In the present report

the increase in insulin-stimulated glucose disposal after diet treatment was primarily accounted for by an enhanced non-oxidative glucose turn-over. Our finding of slightly lower basal insulinaemia after diet treatment is probably not essential to the observed changes in *in vivo* insulin action. Indeed, when Type 2 diabetic patients are treated with insulin or sulfonylurea there is a concomitant increase in diurnal hyperinsulinaemia which does not obstruct an increase in insulin sensitivity [9, 25, 26]. Thus, although hyperinsulinaemia may cause insulin resistance in non-diabetic subjects [27, 28] improved metabolic control in Type 2 diabetes seems to have a greater impact on the insulin sensitivity than the potential negative effects of hyperinsulinaemia.

Our forearm balance data confirm previous findings [29] that hyperglycaemia in the basal (post-absorptive)

Fig. 3. Correlation of the diet-induced change of insulin-mediated glucose disposal during hyperinsulinaemia with weight loss ($r = 0.78$, $p < 0.05$), reduction in fasting plasma glucose level ($r = 0.97$, $p < 0.001$), reduction in plasma fructosamine concentration ($r = 0.77$, $p < 0.05$) and reduction in dietary energy intake ($r = 0.02$, $p = \text{NS}$)

Fig. 4. Scatchard plots of insulin receptor binding to wheat germ agglutinin-purified insulin receptors extracted from skeletal muscle of control subjects (●), Type 2 diabetic patients before diet (○) and after diet (▽). Insert: Basal and insulin-stimulated kinase activity of insulin receptors (given as the rate of phosphorylation of poly(Glu-Tyr(4:1))) from the same groups. Mean values and SEM are shown

state causes an increased glucose uptake in forearm muscle due to 'non-insulin-mediated glucose uptake' which is normal in Type 2 diabetes [30]. Consequently, lower basal plasma glucose levels during diet treatment lead to a normalization of basal forearm glucose uptake. Insulin-stimulated glucose uptake in forearm muscle also tended to normalize after diet treatment. Such data emphasise the importance of skeletal muscle for the observed enhancement of peripheral insulin sensitivity during diet treatment.

Although few patients were studied the finding of a correlation between weight loss and increase in insulin-stimulated glucose turn-over suggests that weight loss per se may be important for diabetic control. Since we found no correlation between reduction in energy intake and increase in insulin-stimulated glucose utilization it may be speculated that reduction of insulin resistance is not solely a question of diminished caloric intake. However, the present results do not justify extensive conclusions on this subject.

Near-normalization of glycaemic control during diet treatment in obese patients with Type 2 diabetes caused no change in insulin receptor binding or kinase activity which were not significantly different from values in the control group. The latter confirms previous findings in studies of insulin receptors from obese Type 2 diabetic patients vs obese control subjects [31, 32]. Similarly, treatment of Type 2 diabetic patients with sulfonylurea caused no change in muscle insulin receptor function [9]. By contrast, insulin receptor kinase activity from adipocytes may improve after diet treatment and weight reduction in Type 2 diabetic patients [3].

It is in accordance with several other studies that the estimate of total GS activity in muscle (measured at saturating G6P concentrations) was similar in Type 2 diabetic patients and control subjects. It is not surprising therefore, that total GS activity did not change during diet-induced

improvement of glycaemic control as already demonstrated by Johnson et al. [33]. This also parallels findings after sulfonylurea treatment of Type 2 diabetic patients [9]. Also in line with previous studies [33–35] is our finding that the activity indices for GS ($A_{0.5}$ for G6P) in the basal state were similar in control subjects and diabetic patients (both before and after diet treatment). To further characterize the GS enzyme we wanted to study the rate with which it became activated due to interference with the endogenous phosphatases [36]. The present finding that GS conversion rates did not change after improved glycaemic control in diabetic patients might suggest that phosphatase activity is less dependent on the metabolic state than is GS activity. Kida et al. [37] found in muscle biopsies obtained in the basal state that the activity index for GS was normal whereas the GS-phosphatase activity was decreased. We saw a similar tendency, although statistical significance was not reached. However, since the GS is probably phosphorylated differently during hyperinsulinaemia before and after diet treatment there is a possibility that differences in conversion rates might not be comparable. To circumvent this problem other groups have added exogenous GS [37]. However, such assay preparations still contain considerable amounts of endogenous GS which interferes with measurements (Bak, J. F. unpub-

Fig. 5. Glycogen synthase activity in skeletal muscle biopsies obtained in the basal state and during hyperinsulinaemic, euglycaemic clamp given as: (from upper to lower) total glycogen synthase (GS) activity, half-maximal activation constant for glucose 6-phosphate ($A_{0.5}$ for G6P), and conversion rate of GS i.e. the rate of accumulation of G6P independent from (I form) during 10 min of incubation in the absence of fluoride. Data are from seven obese control subjects (□), seven obese Type 2 diabetic patients before diet treatment (▨) and after diet treatment (■). Mean values $\pm$ SEM are shown; * $p < 0.03$

lished results). More precise estimation of GS phosphatase activity must await development of more appropriate techniques.

Insulin exposure conveys a stimulation of muscle GS through dephosphorylations which lead to a decrease in $A_{0.5}$ for G6P. It is well known that insulin resistance in normoglycaemic subjects is associated with insulin resistant activation of GS [8]. In the present study insulin activation of GS in Type 2 diabetic patients improved after non-pharmacological (dietary) treatment. Sulfonylurea treatment of untreated Type 2 diabetic patients (non-responders to dietary intervention) had similar effects [9].

According to the protocol, the basal step of the clamp was more hyperglycaemic in Type 2 diabetic patients before than after diet. The importance of hyperglycaemia for overall insulin sensitivity and insulin activation of muscle GS has attracted much interest in recent years. It appears that acute elevations of plasma glucose do not interfere with insulin activation of muscle GS in normoglycaemic healthy control subjects [36, 38] whereas chronic hyperglycaemia in diabetic patients may be important for insulin stimulation of GS [34, 35, 39]. Thus, over-night normalization of plasma glucose (through a low-dose insulin infusion) in Type 2 diabetic patients caused an inactivation of GS both in the basal state and during insulin stimulation [34]. Some years ago it was demonstrated that catecholamines may be released in variable amounts in response to an acute fall in plasma glucose from hyperglycaemia to euglycaemia in diabetic subjects [40, 41]. However, there was no proportionality between the fall in plasma glucose and catecholamine release and the findings were not confirmed in a more recent report [23].

Our finding that caloric intake may influence insulin-stimulated glucose disposal and muscle GS activation is in accordance with findings in normoglycaemic healthy volunteers [4]. However, in contrast to the present study Johnson et al. [33] have reported an absence of insulin activation of GS in muscle from Type 2 diabetic subjects both before and after dietary control of hyperglycaemia. One explanation for this discrepancy may be that we use $A_{0.5}$ for G6P based on several measurements of fractional velocities as an estimate of GS activity. Fractional velocities have been reported to be more sensitive than activity ratios (absence of G6P/presence of 10 mmol/l G6P) for determination of changes in GS activity [42]. Furthermore, we only re-studied a selected group of Type 2 diabetic patients who responded to diet therapy.

Newly-diagnosed patients with Type 2 diabetes who respond to non-pharmacological treatment may be in the same metabolic state as before the development of clinical diabetes. Recent reports from first-degree relatives of patients with Type 2 diabetes suggest that impaired insulin stimulation of glucose storage [43] and reduced insulin stimulation of GS activity in muscle [44] may be primary defects in this disease. However, the present data suggest that defects in insulin action may be reversible during non-pharmacological treatment and that diet habits may be of a primary importance as well. To address this crucial question the effects of diet intervention in non-diabetic first-degree relatives to Type 2 diabetic patients should be examined.

In conclusion, newly-diagnosed obese Type 2 diabetic patients showed an impaired insulin stimulation of glucose disposal, non-oxidative glucose metabolism, and forearm glucose uptake. In muscle biopsies an abnormally low insulin activation of GS was found whereas there were no defects in insulin receptor kinase activities. Diet-induced improvement of glycaemic control tended to normalize the defects, without changing the rate by which GS converts to more active forms.

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Dr. J. F. Bak
Medical-Endocrinological Department C
Aarhus Amtssygehus
DK-8000 Aarhus C
Denmark