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Inhibition of muscle glycogen synthase activity and non-oxidative glucose disposal during hypoglycaemia in normal man

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Summary The purpose of the present study was to evaluate the role of muscle glycogen synthase activity in the reduction of glucose uptake during hypoglycaemia. Six healthy young men were examined twice; during 120 min of hyperinsulinaemic (1.5 mU · kg^{-1} · min^{-1}) euglycaemia followed by: 1) 240 min of graded hypoglycaemia (plasma glucose nadir 2.8 mmol/l) or 2) 240 min of euglycaemia. At 350–360 min a muscle biopsy was taken and indirect calorimetry was performed at 210–240 and 330–350 min. Hypoglycaemia was associated with markedly increased levels of adrenaline, growth hormone and glucagon and also with less hyperinsulinaemia. During hypoglycaemia the fractional velocity for glycogen synthase was markedly reduced; from 29.8 ± 2.3 to 6.4 ± 0.9 %, $p < 0.05$. Total glucose disposal was decreased during hypoglycaemia (5.58 ± 0.55 vs 11.01 ± 0.75 mg · kg^{-1} · min^{-1} (euglycaemia); $p < 0.05$); this was primarily due to a reduction of non-oxidative glucose disposal (2.43 ± 0.41 vs 7.15 ± 0.7 mg · kg^{-1} · min^{-1} (euglycaemia); $p < 0.05$), whereas oxidative glucose disposal was only suppressed to a minor degree. In conclusion hypoglycaemia virtually abolishes the effect of insulin on muscle glycogen synthase activity. This is in keeping with the finding of a marked reduction of non-oxidative glucose metabolism. [Diabetologia (1996) 39: 226–234]

Key words Hypoglycaemia, counter-regulation, glucose disposal, muscle glycogen synthase activity, glucose mass effect.

Hypoglycaemic episodes represent a major treatment-induced problem [1–5]. Severe hypoglycaemia is more frequent in patients receiving intensive insulin treatment, and the recent results from the Diabetes Control and Complications trial (DCCT) [6], proving the benefit of strict metabolic control on late diabetic complications, may be anticipated to enhance this problem.

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Abbreviations: HGP, Hepatic glucose production; Rd, glucose disposal; GH, growth hormone; 3-OHB, 3-hydroxybutyrate; G 6-P, glucose 6-phosphate; NEFA, non-esterified fatty acids; PP-1, phosphatase-1; Ra, rate of appearance; Rd-nonox, non-oxidative glucose disposal; Rd-ox, oxidative glucose disposal; A0.5, half-maximal activity.

In studies involving short-term and more prolonged insulin infusion as well as the pituitary-adrenal-pancreatic-clamp technique [7–10] the present concept of hypoglycaemic counter-regulation has emerged; within minutes of lowering the plasma glucose level the combined effects of suppressed endogenous insulin secretion, increased secretion of glucagon and epinephrine, as well as glucose autoregulation [11], lead to enhanced hepatic glucose production (HGP) and reduced glucose disposal (Rd). With more prolonged (2 h) hypoglycaemia, the effects of increased growth hormone (GH) and cortisol secretion also become evident. Increased substrate availability, serving as an independent fuel source (non-esterified fatty acids [NEFA], ketones) and as gluconeogenic precursors (lactate, amino acids, glycerol) also participate [12–16].

HGP is known to be increased both due to increased glycogenolysis and increased gluconeogene-

sis [15–17], whereas the mechanism behind the suppressed glucose utilisation, which becomes more important with more prolonged hypoglycaemia [9], is less well described. In recent studies we and others have demonstrated that muscle glucose uptake is dramatically reduced during hypoglycaemia in healthy man [18–20]. However, the specific cellular events involved in this phenomenon require further examination.

To clarify this issue we employed hyperinsulinaemia-induced hypoglycaemia combined with muscle biopsies and indirect calorimetry to assess muscle glycogen synthase activity, intramuscular glucose and glucose 6-phosphate (G 6-P) content and the relative importance of oxidative- and non-oxidative glucose disposal in the defence against hypoglycaemia.

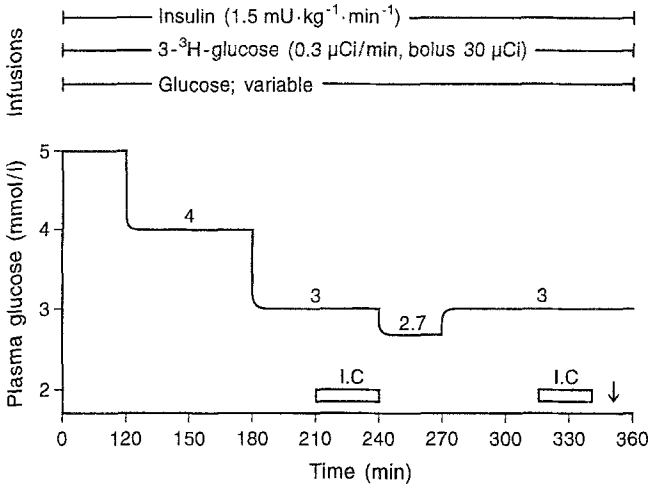

Fig. 1. Study design. I.C., indirect calorimetry; arrow indicates muscle biopsy

Subjects and methods

Subjects. Six healthy young volunteers participated in the study. Mean age was 25 ± 1.7 years; BMI, 22.2 ± 0.9 kg/m^2. The study protocol was approved by the local ethical committee, and all subjects gave informed consent.

Experimental protocol (Fig. 1)

Each subject underwent two studies, i.e. hypoglycaemic and euglycaemic, with an interval of approximately 1 month.

Both studies commenced at 08.00 hours after an overnight fast. Two catheters were placed; one in a heated dorsal hand vein for sampling of arterialized blood, another in the contralateral antecubital vein for infusions. Throughout both studies, human insulin (Insulin Actrapid human; Novo-Nordisk, Copenhagen, Denmark) was infused at a rate of 1.5 mU · kg^{-1} · min^{-1} and (3-^{3}H-)glucose (Du Pont New-England Nuclear, Boston, Mass., USA) in a primed (30 μCi) continuous (0.3 μCi/min) manner, in order to determine glucose turnover. 3-^{3}H-glucose batches contained no contaminating radiochemicals as assessed by HPLC.

Glucose clamp. Glucose (55 and 200 g/l) was infused at variable rates to obtain the desired plasma glucose level. Throughout the control study and during the first 120 min of the hypoglycaemic study plasma glucose was clamped at 5 mmol/l, to allow the isotope to equilibrate (these first 2 study hours are hereafter designated the equilibration period). In the hypoglycaemic study, hypoglycaemia was then induced (by reducing exogenous glucose administration) aiming at a nadir of 2.8 mmol/l (Fig. 1).

Blood sampling. Plasma glucose was measured every 5 min by a glucose analyser (Beckman Instruments, Palo Alto, Calif., USA). Blood for determination of glucose, glucose-specific activity, insulin, C-peptide, GH, glucagon, epinephrine, norepinephrine, NEFA and blood metabolites was drawn as shown in Figures 2 and 3.

Muscle biopsy. At the end of each study (345–360 min) a muscle biopsy was obtained from the vastus lateralis muscle as previously described [21].

Indirect calorimetry. Energy expenditure and respiratory exchange ratio were measured at 210–240 and 320–340 min.

A computerized, open circuit system was employed to measure gas exchange across a 25-litr canopy (Deltatrac, Datex Instrumentarium Inc., Helsinki, Finland). The monitor determines carbon dioxide production and oxygen consumption by multiplying dry air flow through the canopy with alterations in gas concentration over the canopy. Estimated net glucose and lipid oxidation rates were calculated from the above measurements and protein oxidation rates were estimated from the urinary excretion rates of urea [22]. Net non-oxidative glucose disposal (Rd-nonox) was determined by subtracting oxidative glucose disposal (Rd-ox) from total Rd, measured isotopically.

Analytical methods

Plasma glucose was measured in duplicate immediately after sampling (Beckman Instruments). Plasma glucagon was determined by radioimmunoassay as previously described with the modification that (PEG = poly ethylen glycol) was used for separation prior to determination and that plasma was extracted with ethanol [23]. Serum insulin was measured using a monoclonal sandwich assay with no cross-reaction with proinsulin or major split-products of insulin [24]. Serum C-peptide was assayed employing a commercial kit (Immunonuclear Corp., Stillwater, Minn., USA) while serum GH was measured using an immunofluorometric sandwich assay with two monoclonal antibodies (Delfia hGH kit; Wallac Oy, Turku, Finland). Catecholamines were determined by electrochemical detection following HPLC [25]. Serum NEFA were determined by a colorimetric method employing a commercial kit (Wako Chemicals, Neuss, Germany). Blood 3-hydroxybutyrate (3-OHB), glycerol, lactate and alanine were assayed by automated enzymatic fluorometric methods [26]. For determination of tritiated glucose activity, plasma was deproteinized using 0.3 mol/l Ba $(OH)_2$ and 5 % $ZnSo_4$ after which the supernatant was evaporated under vacuum, resuspended in distilled water and counted by a liquid scintillation counter after the addition of 5 ml Aqualuma Plus (Lumbac, Schaesburg, the Netherlands).

Muscle enzyme analysis. Glycogen synthase activity was measured as described previously [27] using 0.13 mmol/l UDP-U-

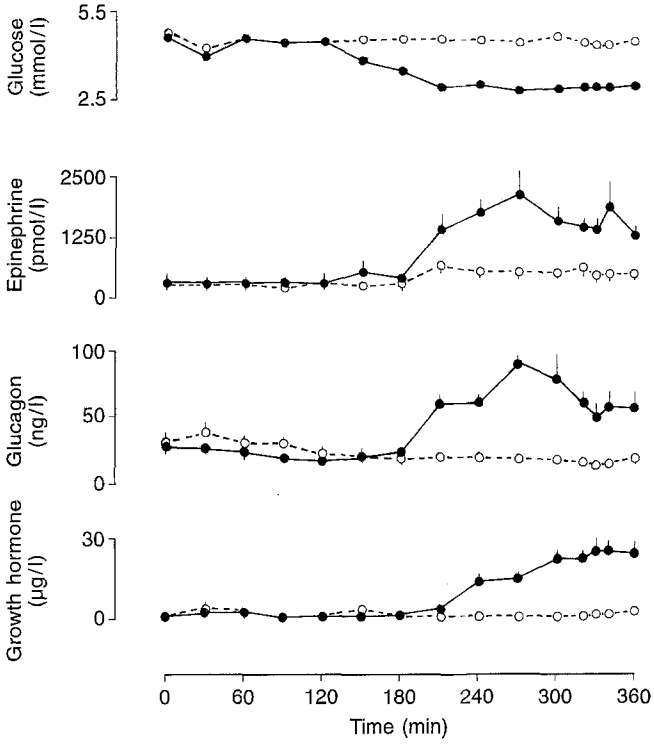

Fig. 2. Plasma glucose, serum epinephrine, plasma glucagon, serum GH concentrations during a hyperinsulinaemic, hypoglycaemic- (●) and euglycaemic clamp (○) in six healthy subjects

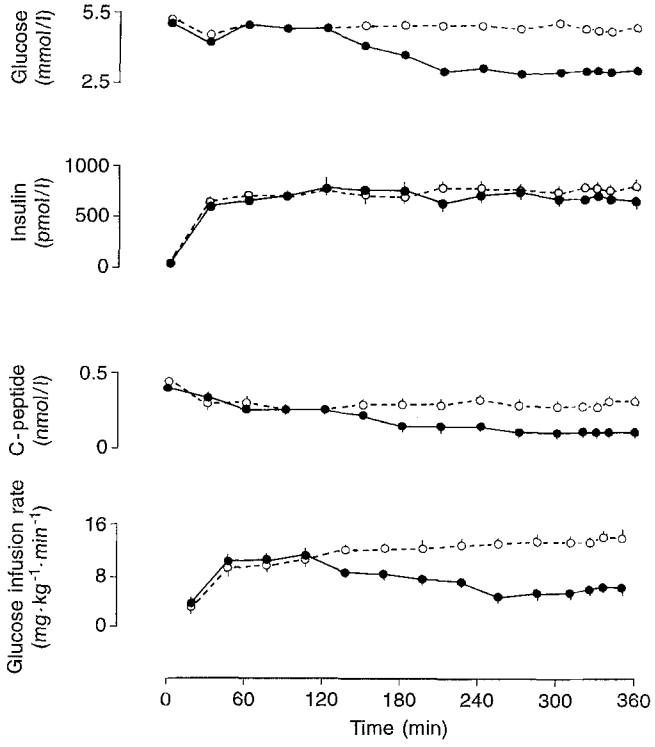

Fig. 3. Plasma glucose, serum insulin and C-peptide concentrations and glucose infusion rates during a hyperinsulinaemic, hypoglycaemic- (●) and euglycaemic clamp (○) in six healthy subjects

^{14}C-glucose (DuPont-New England Nuclear), 0.67 % (w/v) glycogen, and 0–6.7 mmol/l G 6-P (final concentrations). In this context, 1 U of glycogen synthase activity equals the incorporation of 1 nmol of UDP-glucose into glycogen per min. Total glycogen synthase activity is defined as the activity in the presence of a saturating concentration (6.7 mmol/l) of G 6-P. Fractional velocities were calculated as glycogen synthase activity in the presence of a subsaturating concentration of G 6-P (0.07 mmol/l) divided by glycogen synthase activity in the presence of 6.7 mmol/l G 6-P. The concentration of G 6-P giving half-maximal activity of the glycogen synthase (A0.5 for G 6-P), representing a different mode of expressing glycogen synthase activity, was calculated using a Hill plot [28].

G 6-P, glucose and glycogen determination. G 6-P glucose- and glycogen content in the muscle biopsy were estimated according to Lowry and Passonneau [29]. Intramuscular glucose was corrected for glucose content in the extracellular space by subtracting the estimated glucose content in the extracellular space (0.3 l/kg dry weight × plasma glucose) from the total glucose content [30]. It is recognized that this is an estimate, as the exact extent of extracellular water is uncertain, especially during hypoglycaemia.

Statistical analysis

The rates of total glucose appearance (Ra) and total Rd were determined from tritiated glucose data in samples taken every 15 min throughout the study. The values were calculated according to the non-steady-state equations of Steele [31] as modified by DeBodo et al. [32]. A distribution volume of 220 ml/kg and a pool fraction of 0.65 was employed. HGP was calculated by subtracting the glucose infusion rate (GIR) from Ra.

All values are expressed as mean ± SEM. Two-way analysis of variance with repeated measures for two factors (time and treatment) was employed a priori to test for changes with time and between treatments. If this test revealed significant differences the Student's t-test for paired data was used to further assess differences between experiments.

Results

Glucose and hormones (Figs. 2, 3). Basal plasma glucose levels (t = 0) were similar in the two studies (5.1 ± 0.2 vs 5.3 ± 0.1 mmol/l, euglycaemia vs hypoglycaemia). Likewise plasma glucose levels did not differ during the first 2 h of the study (t, 0–120 min = equilibration period). Throughout the rest of the hypoglycaemic study plasma glucose levels were kept lower, reaching a mean minimum of 2.9 ± 0.1 mmol/l after 270 min (Fig. 2).

Basal and equilibration levels of epinephrine, norepinephrine, GH and glucagon were similar in the two studies. During euglycaemia epinephrine levels remained stable with a mean value of 589 ± 164 pmol/l at t 240–360 min. During hypoglycaemia levels increased significantly ($p < 0.05$), reaching a maximum value of 2123 ± 486 pmol/l at 270 min.

GH levels were stable in the euglycaemic study with a mean value of 1.5 ± 0.5 µg/l at 240–360 min.

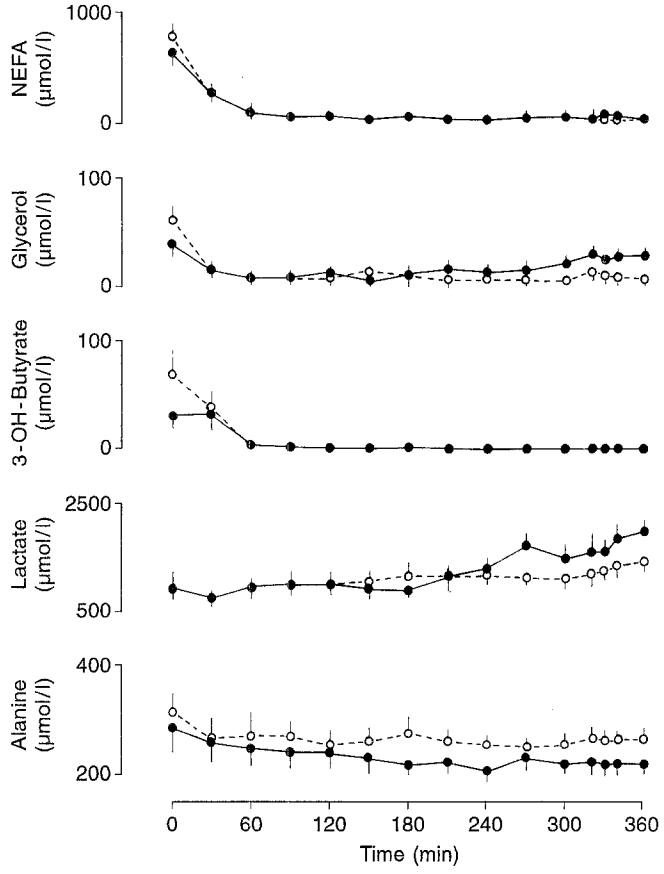

Fig. 4. Concentrations of serum NEFA, blood glycerol, 3-OHB, lactate and alanine during a hyperinsulinaemic, hypoglycaemic- (●) and euglycaemic clamp (○) in six healthy subjects

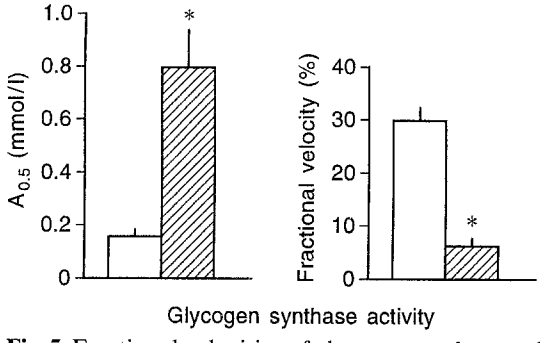

Fig. 5. Fractional velocities of glycogen synthase at 0.07 mmol/l G 6-P in muscle biopsies and corresponding half-maximal activation constants (A 0.5) for G 6-P during a hyperinsulinaemic, euglycaemic- (□) and hypoglycaemic clamp (▨) in six healthy subjects. Values are mean ± SEM. * $p < 0.05$

During hypoglycaemia levels increased sharply to 14.0 ± 3.8 µg/l when plasma glucose was lowered to 3 mmol/l and stabilized at 21.2 ± 0.01 µg/l during the last 2 h ($p < 0.05$).

Glucagon levels were reduced to a similar extent during the equilibration period. In the euglycaemic study levels stabilized thereafter (17 ± 3 ng/l, at t, 240–360 min). During hypoglycaemia glucagon levels increased significantly reaching a maximum of 88 ± 6 µg/l at 270 min ($p < 0.05$).

Table 1. Individual values of fractional velocity (FV) of glycogen synthase activity at 0.07 mmol/l G 6-P in muscle biopsies during euglycaemia and hypoglycaemia

Subject	FV (%) Euglycaemia	FV (%) Hypoglycaemia
1	25.7	7.3
2	34.2	5.7
3	34.5	6.3
4	23.9	9.8
5	36.0	3.4
6	24.3	6.3

Basal insulin levels were similar at 37 ± 4 and 36 ± 4 pmol/l (euglycaemia vs hypoglycaemia, NS); insulin infusion caused levels to increase to a mean level of 756 ± 30 pmol/l during the last 2 h in the euglycaemic study, whereas during hypoglycaemia, levels were slightly lower (684 ± 60 pmol/l, $p < 0.05$).

During insulin infusion an initial reduction in C-peptide levels was seen in both studies (0.43 ± 0.03 nmol/l at basal to 0.26 ± 0.03 nmol/l at 120 min during euglycaemia). Hypoglycaemia caused C-peptide levels to be further reduced after 240 min (0.13 ± 0.03 nmol/l), whereas levels were stable in the control study (0.3 ± 0.03 nmol/l, $p < 0.05$).

Gluconeogenic precursors and lipid intermediates (Fig. 4). From a baseline level of 774 ± 115 vs 642 ± 108 µmol/l (euglycaemia vs hypoglycaemia, NS) insulin infusion suppressed NEFA levels equally (43 ± 1 vs 68 ± 18 µmol/l, euglycaemia vs hypoglycaemia, NS).

Insulin infusion caused a similar initial suppression of glycerol levels to 8 ± 4 vs 12 ± 4 µmol/l (NS). In the euglycaemic study values then stabilized (8 ± 3 µmol/l, during the last 2 study hours), whereas during hypoglycaemia there was a small breakthrough in glycerol concentration (26 ± 5 µmol/l, $p < 0.05$).

Insulin infusion totally suppressed 3-OHB levels in both studies.

During hyperinsulinaemic euglycaemia, blood lactate concentrations increased slightly but significantly with time from a basal level of 873 ± 106 to 1243 ± 129 µmol/l during the last 2 h ($p < 0.05$). During the last 2 h of hypoglycaemia, a more pronounced increase occurred (1675 ± 220 µmol/l, $p < 0.05$).

In both studies alanine levels fell slightly with time and levels did not differ between the two studies.

Muscle enzymes (Fig. 5, Table 1). Total activities of glycogen synthase (measured at saturating levels of G 6-P and physiologic levels of UDP-glucose) were similar (44.8 ± 3.2 vs 49.5 ± 4.2 U/mg protein, hypoglycaemia vs euglycaemia).

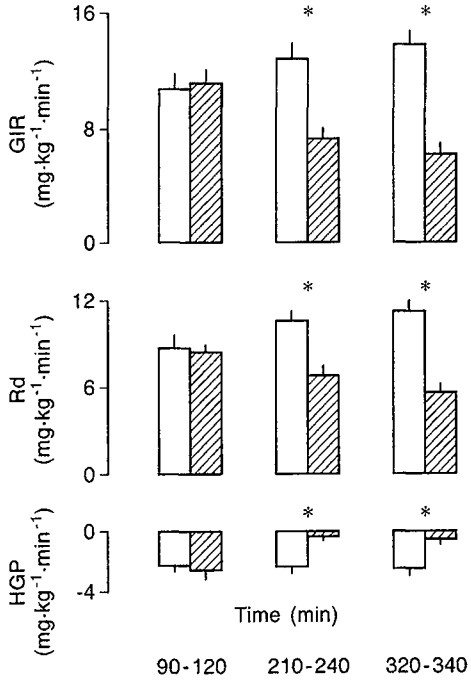

Fig. 6. Rates of glucose infusion (GIR), total glucose disappearance (Rd) and HGP during a hyperinsulinaemic, euglycaemic- (□) or hypoglycaemic clamp (▨) in six healthy subjects. Values are mean ± SEM. * $p < 0.05$

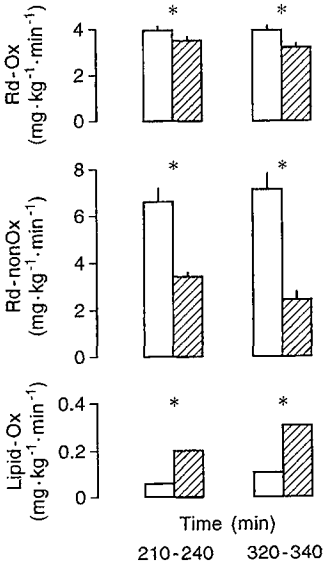

Fig. 7. Rates of oxidative glucose disposal (Rd-ox), non-oxidative glucose disposal (Rd-nonOx) and lipid oxidation (lipid-ox) during a hyperinsulinaemic, euglycaemic- (□) or hypoglycaemic clamp (▨) in six healthy subjects. Values are mean ± SEM. * $p < 0.05$

Hypoglycaemia inhibited the fractional velocity (at 0.07 mmol/l G 6-P) by 80% from 29.8 ± 2.3 to 6.4 ± 0.9% ($p < 0.05$). Likewise the sensitivity of the glycogen synthase to G 6-P was reduced by hypoglycaemia as shown by an increase in A0.5 for G 6-P from 0.16 ± 0.02 to 0.8 ± 0.15 mmol/l ($p < 0.01$).

G 6-P, glucose and glycogen. The muscle content of G 6-P tended to be higher during hypoglycaemia; 0.68 ± 0.12 vs 0.29 ± 0.09 mmol/kg dry weight ($p = 0.06$). Due to technical problems, it was only possible to determine G 6-P in five patients. The difference in calculated intramuscular glucose content (1.49 ± 0.32 vs 1.16 ± 0.39 mmol/kg dry weight, hypoglycaemia vs euglycaemia, $p = 0.14$) did not reach statistical significance. Likewise it was not possible to detect any difference in glycogen content (567 ± 32 vs 535 ± 50 mmol/kg dry weight, hypoglycaemia vs euglycaemia; p = NS).

Glucose kinetics (Fig. 6). The glucose infusion rate was significantly lower throughout the hypoglycaemic period, reaching a minimum at 240–270 min (4.85 ± 1.15 vs 12.99 ± 0.97 mg · kg^{-1} · min^{-1}, $p < 0.001$; hypoglycaemia vs euglycaemia).

Rd increased from a level of 8.70 ± 0.91 mg · kg^{-1} · min^{-1} during the last 30 min of the equilibration period and stabilized at a level of 11.01 ± 0.75 mg · kg^{-1} · min^{-1} during the last 2 h of euglycaemia. During hypoglycaemia Rd was inhibited to a mean of 5.58 ± 0.55 mg · kg^{-1} · min^{-1} during the last 2 h ($p < 0.05$).

Ra increased to a similar extent during the equilibration period. During euglycaemia levels then stabilized with a mean of 11.03 ± 0.8 mg · kg^{-1} · min^{-1} during the last 2 h whereas during hypoglycaemia a minimum was reached at 320 min (5.48 ± 0.47 vs 11.99 ± 0.75 mg · kg^{-1} · min^{-1}, hypoglycaemia vs euglycaemia), whereafter levels increased slightly (6.32 ± 0.61 mg · kg^{-1} · min^{-1} at 340–360 min) $p < 0.05$).

HGP was suppressed to negative values in both studies. Equilibration levels (90–120 min) were similar (–2.57 ± 0.62 vs –2.16 ± 0.39 mg · kg^{-1} · min^{-1}) During euglycaemia HGP continued to be excessively suppressed, whereas HGP was stimulated by hypoglycaemia (–0.56 ± 0.36 vs –2.73 ± 0.44 mg · kg^{-1} · min^{-1} (320–340 min); $p < 0.05$).

Indirect calorimetry (Fig. 7). Energy expenditure as calculated from oxygen consumption was similar in the two studies (72.3 ± 2.4 vs 76.9 ± 2.4 cal/h; $p > 0.05$).

Non-protein respiratory exchange ratio was slightly but significantly lower during early – as well as late – phase hypoglycaemia (0.9 ± 0.01 vs 0.99 ± 0.01; 210–240 min; $p < 0.05$) and did not change with time in the two studies.

Calculated glucose oxidation was slightly lower during hypoglycaemia (3.2 ± 0.1 vs 3.9 ± 0.2 mg · kg^{-1} · min^{-1}; $p < 0.05$, at 320–340 min) and levels remained stable during the separate studies. However, calculated Rd-nonox was found to be profoundly inhibited by hypoglycaemia, and this was even more pronounced in the later phase (3.36 ± 0.54 vs 6.62 ± 0.67 mg · kg^{-1} · min^{-1}; $p < 0.001$, 210–240 min and

2.43 ± 0.41 vs 7.15 ± 0.7 mg · kg^{-1} · min^{-1}; $p < 0.001$, 320–340 min).

Protein oxidation was similar and averaged 0.8 mg · kg^{-1} · min^{-1}. Insulin infusion suppressed lipid oxidation to a greater extent during euglycaemia when compared to hypoglycaemia (0.11 ± 0.05 vs 0.30 ± 0.07 mg · kg^{-1} · min^{-1}; $p < 0.05$; 320–340 min).

Discussion

This study focuses on the role of glycogen synthase activity in the reduction of glucose uptake during hypoglycaemia. We found total Rd to be reduced to about 50 % of euglycaemic values. The activity of glycogen synthase in skeletal muscle was reduced by 80 % during hypoglycaemia, i.e. to a level comparable to that found in the basal state [21], and Rd-nonox was substantially suppressed. Due to the limitations of the isotope dilution technique in its present form, "physiologically impossible" negative values for HGP occur. Therefore, these data should be interpreted qualitatively rather than quantitatively. Bearing this in mind, hypoglycaemia seemed to exert a stimulating effect on HGP.

Under conditions of euglycaemic hyperinsulinaemia, muscle has been shown to account for up to 85 % of the amount of glucose to be metabolized [33]. In recent studies, we and others [18, 19] have shown forearm glucose uptake to be dramatically reduced during hyperinsulinaemia-induced hypoglycaemia, and the present study implicates a pivotal role for the profoundly reduced muscle glycogen synthase activity in this condition.

The role of decreased mass action of glucose [34] also demands consideration. Hyperglycaemia (glucose per se) has been suggested to stimulate Rd-nonox and to a lesser extent Rd-ox [35], whereas no such stimulating effect has been demonstrated on glycogen synthase activity [36]. Even though these results cannot be directly extrapolated to the hypoglycaemic setting of the present study, the fact that Rd decreased even before any significant counter-regulatory hormonal release was evident (at a plasma glucose level of 4 mmol/l) point towards an independent effect of a reduction in the plasma glucose level to reduce glucose uptake. This is supported in a recent study by Capaldo et al. [19] demonstrating a reduced glucose mass effect in forearm experiments.

Non-oxidative glucose metabolism has several possible pathways: 1) formation of glycogen, 2) anaerobic glycolysis and subsequent lactate formation, 3) conversion into lipids. The amount of lactate released from muscle in response to insulin is small, even during moderate hypoglycaemia [18, 19, 33], and presumably only small amounts of glucose are converted into lipids [37], and the major fate for glucose taken up into muscle has been ascribed to glycogen synthesis. In support of this, a positive correlation has been found between glycogen synthase activity and Rd-nonox [38, 39] and by using nuclear magnetic resonance spectroscopy. Shulman et al. [40] have shown that under conditions of euglycaemic hyperinsulinaemia glycogen synthesis represents the primary pathway for Rd-nonox.

Several of the participants in the counter-regulatory set-up could be responsible for the inhibitory effect of hypoglycaemia on glycogen synthase activity and Rd-nonox.

Epinephrine acts in defence against short-term as well as long-term hypoglycaemia and has been demonstrated to antagonize insulin action on muscular tissue [41].

Insulin stimulation of phosphatase-1 (PP-1) which activates glycogen synthase has been shown to be a result of a phosphorylation at site-1 of the glycogen-binding G-subunit of PP-1 [42]. In contrast, the cyclic AMP-dependent kinase (activated by e.g. catecholamines) phosphorylates both sites-1 and -2 of the G-subunit, and activates the specific inhibitor of PP-1, thereby inactivating PP-1 [42, 43]. It is in agreement with this observation to hypothesize that insulin stimulation of muscle glycogen synthase may be blocked by elevated plasma epinephrine levels during hypoglycaemia.

In a study where epinephrine was infused during euglycaemic hyperinsulinaemia Raz et al. [44] found that epinephrine inhibits insulin-mediated glycogenesis. This was associated with an inactivation of glycogen synthase and activation of glycogenolysis, which is in agreement with our results. They found an increase in G 6-P, which inhibits hexokinase and thereby glucose uptake, and enhances glycolysis.

In the present study the G 6-P content was also increased during hypoglycaemia and even though the difference in calculated intracellular glucose content did not reach statistical significance, this fits well with the inhibition of glycogen synthase activity and non-oxidative glucose metabolism. We could not demonstrate increased glycolysis when measuring Rd-ox; whether this could be explained by the hypoglycaemia-associated impairment of glucose mass action and subsequent lack of oxidation is unclear. Studies on isolated intact rat muscle support the role of epinephrine in inhibition of insulin-mediated glycogen synthesis and activation of glycogenolysis through activation of glycogen phosphorylase [45, 46].

In a model of prolonged (8-h) hypoglycaemia and relatively low-dose insulin infusion (0.4 mU · kg^{-1} · min^{-1}), Fanelli et al. [47] have demonstrated that a significant part of the catecholamine-induced suppression of Rd may be due to increased lipolysis, leading to increased levels of NEFA and glycerol. In the present study the higher insulin infusion rate

leading to suppression of NEFA levels presumably prevented indirect mediation [48, 49] from playing any major role (a notion supported by the very modest elevation of lipid-oxidation during hypoglycaemia).

Concerning the role of GH and cortisol, GH has been found to inhibit insulin-mediated activation of glycogen synthase in skeletal muscle [21] and, also in agreement with the findings in the present study, to inhibit glucose oxidation in favour of lipid oxidation. The effect of GH on muscle has been shown to be delayed by approximately 2 h and part of its effect has been suggested to be direct, rather than dependent on lipolysis [50].

Previously cortisol was thought to play only a minor role in short-term counter-regulation. However, in a study of prolonged hypoglycaemia, De Feo et al. [51] demonstrated a significant cortisol-induced stimulation of HGP after 3 h and a significant inhibition of glucose utilisation after 4 h. Some of these effects might be secondary to stimulation of lipolysis. Apart from the indirectly mediated effects, cortisol has been shown to reduce the speed by which insulin activates glucose transporters and also to mediate the effect of insulin at a post-binding level [52, 53]. In vitro studies have shown long-term dexamethasone treatment (14 days) to be associated with an inhibition of insulin-stimulated glycogen synthesis and a decrease in glycogen synthase activity in muscle. The reduction in glycogen synthesis was associated with increased NEFA levels [54].

Calculation of glucose-, lipid- and protein-oxidation from indirect calorimetry data is based on a number of assumptions, and in particular ongoing lipogenesis and gluconeogenesis may influence results [22], necessitating cautious interpretation. In a dose-response study on the relationship between plasma insulin concentration and total Rd, Rd-ox and Rd-nonox [55], Rd-nonox was found to represent the major route of glucose disposal during euglycaemic hyperinsulinaemia, and glucose oxidation was saturated at lower plasma insulin concentrations than glucose storage. In the present study hypoglycaemia especially compromised the route of Rd-nonox, a fact which seems logical, considering that insulin exerts its major influence here.

Recently, Shamoon et al. [56] demonstrated muscle glycogen synthase activity to be suppressed and G 6-P content to be increased in non-insulin-dependent diabetic subjects during insulin-induced hypoglycaemia, placing skeletal muscle in a key position in counter-regulation in these subjects. In the control subjects, however, only a minor increase in G 6-P content was found and glycogen synthase activity was unsuppressed. This apparent discrepancy between both our results is probably explained by the presence of higher insulin levels in the study by Shamoon et al. [56] and also it cannot be excluded that some suppression of glycogen synthase activity would have been unmasked if a constant euglycaemic control situation had been present. Our results are in agreement with those of a very recent study by Cohen et al. [20], demonstrating decreased glycogen synthesis in skeletal muscle during mild hypoglycaemia and physiological hyperinsulinaemia in healthy subjects.

The small difference in insulin levels (684 ± 60 vs 756 ± 30 pmol/l; $p < 0.05$) in our study cannot account for the pronounced suppression of glycogen synthase activity and non-oxidative glucose disposal [36, 55]. However, the difference was slightly greater than would have been expected from suppression of endogenous insulin production, and could possibly reflect an elevated clearance of insulin during hypoglycaemia, even though this to our knowledge has not previously been described.

In conclusion, in accordance with the finding of a prominent reduction of Rd-nonox our data suggest that hypoglycaemia virtually abolishes the effect of supraphysiological insulin exposure on muscle glycogen synthase activity.

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