Comparison of the Priming Effects of Pulsatile and Continuous Insulin Delivery on Insulin Action in Man

C.H. Courtney, A.B. Atkinson, C.N. Ennis, B. Sheridan, and P.M. Bell

Insulin is normally secreted in man in regular pulses every 5 to 15 minutes. Disordered pulsation has been demonstrated in several insulin-resistant states and it is unclear whether this represents a primary beta-cell defect contributing to impairment of peripheral insulin action or rather is a consequence of insulin resistance. Basal or near basal insulin administration by pulsatile infusion augments hypoglycemic effect and improves insulin-mediated glucose uptake compared with insulin by continuous infusion. To date no study has examined whether normal basal insulin pulsatility is required to preserve subsequent insulin sensitivity during hyperinsulinemia. We studied the effect of overnight pulsatile versus continuous basal insulin on a subsequent hyperinsulinemic euglycemic clamp. Nineteen normal volunteers (male:female ratio, 17:2; mean age ± SEM, 26.1 ± 2.3 years) were studied on 2 occasions each. Endogenous insulin secretion was inhibited by octreotide (0.43 μg $kg^{-1} \cdot h^{-1}$) and replaced overnight at 5.4 mU $kg^{-1} \cdot h^{-1}$ either by continuous infusion or in 2-minute pulses every 13 minutes (n = 10) or every 7 minutes (n = 9). Glucagon was replaced at physiological concentration by continuous infusion (30 ng $\cdot$ $kg^{-1} \cdot h^{-1}$). Venous plasma glucose overnight was not significantly different between the pulsatile and continuous protocols. After discontinuing the overnight insulin infusion, insulin action was assessed during a hyperinsulinemic euglycemic clamp (1 mU $kg^{-1} \cdot h^{-1}$). Glucose infusion rates at steady-state during the hyperinsulinemic clamp were similar between continuous and both frequencies of pulsatile infusion (continuous 44.6 ± 4.3 μmol $\cdot$ $kg^{-1} \cdot min^{-1}$ *v* 13-minute pulsatile 41.7 ± 5.9 μmol $\cdot$ $kg^{-1} \cdot min^{-1}$, *P* = .27; continuous 34.6 ± 2.5 μmol $\cdot$ kg^{-1} min^{-1} *v* 7-minute pulsatile 41.4 ± 3.2 μmol $\cdot$ $kg^{-1} \cdot min^{-1}$, *P* = .08). We conclude that overnight pulsatile compared with continuous insulin administration has no different effect on subsequent peripheral insulin-mediated glucose uptake. A priming effect cannot therefore explain the previously demonstrated association between endogenous insulin pulse frequency and peripheral insulin action.

INSULIN is secreted in normal individuals in rapid regular pulses with 5- to 15-minute frequency superimposed on slower ultradian oscillations every 80 to 150 minutes.[1] These rapid regular pulses are replaced by brief, irregular pulses in subjects with insulin-resistant states such as obesity, impaired glucose tolerance, and type 2 diabetes mellitus.[2,3] It has been suggested that disorganized insulin secretory pulses may be an early abnormality in the development of insulin resistance as insulin receptor recycling and sensitivity may depend on regular oscillation of insulin.[4,5] Alternatively, peripheral insulin resistance may induce beta-cell defects causing disruption of insulin secretory oscillation. Insulin administered at basal and near basal levels in a pulsatile manner has a more potent hypoglycemic effect than continuous insulin administration.[4,6] We have previously observed in non-obese subjects, both with and without type 2 diabetes, that insulin pulse frequency negatively correlates with insulin mediated glucose uptake,[7,8] which raises the possibility that increased basal pulse frequency might alter peripheral sensitivity to hyperinsulinemia. To investigate this possibility we devised a protocol to deliver either overnight pulsatile or continuous basal insulin and then determine insulin sensitivity using the hyperinsulinemic clamp. Two frequencies of pulsatile insulin were compared with continuous insulin administration as both higher[9] and lower[2] frequencies of basal physiological insulin release have been reported.

From the Regional Centre for Endocrinology and Diabetes, Royal Victoria Hospital, Belfast, UK.

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Address reprint requests to Professor P.M. Bell, Regional Centre for Endocrinology and Diabetes, Royal Victoria Hospital, Belfast, UK BT12 6BA.

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MATERIALS AND METHODS

In the study of continuous versus13-minute pulse frequency, 10 male volunteers were studied on 2 occasions each separated by at least 1 week. Mean age (±SEM) was 21.3 ± 1.4 years and mean body mass index (BMI; ±SEM) 21.9 ± 0.8 kg m^{-2}. In the study of continuous versus 7-minute pulse frequency, 9 volunteers (male:female ratio, 7:2) were similarly studied with age (mean ± SEM) 31.3 ± 4.0 years and BMI (mean ± SEM) 26.2 ± 1.1 kg m^{-2}. Subjects were excluded if they had significant hepatic, renal, or cardiac history or if they were receiving medication known to influence insulin action. Diabetes mellitus in a first-degree relative also precluded study. All patients gave written informed consent to participate in the study and the protocol was approved by the Ethics Committee of The Queen's University of Belfast.

Each subject was admitted to the Regional Centre for Endocrinology and Diabetes, Royal Victoria Hospital at 10 PM having fasted from an evening meal at 6 PM. A left forearm vein was cannulated (21 gauge; Venflon Viggo, Helsingborg, Sweden) for drug administration and a right arm vein cannulated (21 gauge; Venflon Viggo) for venous sampling.

At 11 PM intravenous infusions of octreotide, glucagon, and insulin were commenced. Endogenous insulin and glucagon release was suppressed by an octreotide infusion (0.43 μg $\cdot$ $kg^{-1} \cdot h^{-1}$) with glucagon replaced by continuous infusion (30 ng $\cdot$ $kg^{-1} \cdot h^{-1}$). Insulin was infused continuously on one study night and on the other in a pulsatile fashion with the order being determined by random selection. Regular insulin (Humulin S; Lilly, Basingstoke, UK) was prepared in 100 mL normal saline with human albumin 1.4% added to prevent insulin adsorption and administered on each night at the same total rate (5.4 mU $\cdot$ $kg^{-1} \cdot h^{-1}$) designed to mimic basal concentrations. In the continuous versus 13-minute study, insulin was administered in pulses of 0.585 mU $\cdot$ $kg^{-1} \cdot min^{-1}$ for 2 minutes followed by an interval of 11 minutes on the pulsatile night, while in the continuous versus 7-minute study, pulses of 0.315 mU $\cdot$ $kg^{-1} \cdot min^{-1}$ for 2 minutes followed by an

interval of 5 minutes were given. Pulsatile infusion was achieved using a programmed infusion pump (Graseby 9400 Ambulatory Infusion Pump, Graseby Medical, Watford, Herts, UK). A summary of the study protocol is shown in Fig 1.

From 11 PM to 7 AM, venous samples for glucose were taken hourly and analyzed on a Beckmann Analyser (Beckman RIIC, High Wycombe, UK). Venous samples for insulin, C peptide, and glucagon were taken every 2 hours throughout this period. Between 5:30 AM and 6:30 AM, venous samples for serum insulin were taken immediately before and 3 minutes after each insulin pulse and at the corresponding times on the continuous study nights to demonstrate the presence or absence, respectively, of pulses.

Insulin action was assessed each morning following overnight study using the hyperinsulinemic glucose clamp technique. The right hand was placed in a temperature controlled plexiglass box (Northern Ireland Technology Centre, Automation Division, Queen's University, Belfast, UK) and maintained at 55°C to allow intermittent sampling of arterialized venous blood. A primed continuous infusion of high performance liquid chromatography–purified [3^3H]-glucose (NET 100C; New England Nuclear Research Products Division, Dupont, Stevenage, UK) was administered during a 2-hour equilibration period from 7 AM to 9 AM (−120 minutes to 0 time), after which a 2-hour continuous infusion of insulin (Humulin S, Eli Lilly, Basingstoke, UK) was begun at 1 mU · kg^{-1} · min^{-1}. The priming dose of [3^3H]-glucose was adjusted according to the 7 AM plasma glucose.[10] The overnight infusion of continuous or pulsatile insulin was discontinued at 9 AM with the infusions of octreotide and glucagon continued throughout the clamp. Plasma glucose concentration was maintained at 5.1 mmol/L by an exogenous glucose infusion (20%) and adjusted based on frequent plasma glucose estimations performed on a bedside glucose analyzer. Exogenous glucose was prelabeled with [3^3H]-glucose to match the predicted basal plasma glucose specific activity as described previously[10,11] with the modification that the primed continuous tracer infusion was reduced to 50 % of the basal rate after 20 minutes and to 25% of basal after 40 minutes (in order to maintain tracer steady-state) and was maintained at this rate throughout the remainder of the hyperinsulinemic period.

Analytical Techniques

Venous blood was used for overnight analyses from 11 PM to 7 AM and arterialized venous blood was used for all analyses from 7 AM to 11 AM. Plasma for measurement of glucose specific activity was deproteinized with barium hydroxide and zinc sulfate by the method of Somogyi.[12]

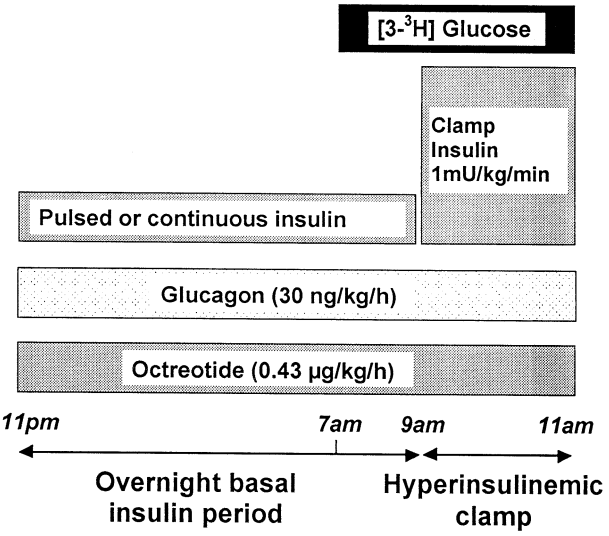

Fig 1. Experimental protocol.

After centrifugation the supernatant was counted in a liquid scintillation spectrometer (Tn-Carb 2000 CA; Canberra Packard, Pangbourne, UK). Aliquots of tracer infusate and labeled exogenous glucose infusion were spiked into nonradioactive plasma and processed in parallel with plasma samples to allow calculation of [3^3H]-glucose infusion rates. Serum insulin was measured by enzyme-linked immunosorbent assay (ELISA) (Abbott Imx, Abbott Laboratories, Berkshire, UK), plasma glucagon was measured by radioimmunoassay (Department of Medicine, Queen's University, Belfast, UK), and plasma C peptide was measured by IMMULITE 1000 C peptide (Diagnostic Products Corp, Los Angeles, CA). Commercially available reagent kits were used for the measurement of serum nonesterified fatty acids (NEFA) (Wako Chemicals, Neuss, Germany), β-hydroxybutyrate (Randox Laboratories, Crumlin, UK), and serum glycerol (Randox Laboratories).

Calculations

The non–steady-state equations of Steele et al[13] as modified by De Bodo et al[14] were used to determine rates of appearance and disappearance of glucose during the periods −30 minutes to 0 time and 90 to 120 minutes, assuming a pool fraction value of 0.65 and an extracellular volume of 190 mL/kg. Rates of infusion of [3^3H]-glucose were calculated as the sum of the tracer infused continuously and the tracer in the labeled exogenous glucose infusion. Rates of endogenous (hepatic) glucose production were then calculated by subtraction of the exogenous glucose rates required to maintain euglycemia from the isotopically determined rates of appearance of glucose.

Statistical Methods

The power of the study, calculated from previous clamp data, gave a 90% chance of detecting a 10% change in insulin action at the 5% level of significance. Significance was assessed using the Student's *t* statistic. Results are expressed as means ± SEM with $P < .05$ considered statistically significant.

RESULTS

Overnight serum insulin, glucagon, growth hormone, plasma C peptide, and glucose levels are shown in Fig 2. Suppression of endogenous insulin secretion was confirmed by reduction of plasma C peptide within 2 hours of the start of the octreotide infusion with C peptide suppression maintained until the end of the study in all cases. Mean serum insulin concentrations overnight were similar during the continuous and pulsatile infusions (continuous *v* 13-minute pulses: 14.9 ± 0.9 mU · L^{-1} *v* 12.6 ± 1.4 mU · L^{-1}, $P = .38$; continuous *v* 7-minute pulses: 19.5 ± 1.1 mU · L^{-1} *v* 21.1 ± 2.1 mU · L^{-1}, $P = .32$). Physiological replacement of glucagon was achieved and did not differ between study nights (continuous *v* 13-minute pulses: 78.4 ± 3.1 ng · L^{-1} *v* 77.3 ± 3.1 ng · L^{-1}, $P = .68$; continuous *v* 7-minute pulses: 85.1 ± 3.9 ng · L^{-1} *v* 86.2 ± 4.1 ng · L^{-1}, $P = .5$). Plasma glucose was measured hourly throughout the night, and in the continuous versus 13-minute study, 4 of 10 subjects had significantly lower plasma glucose levels overall on the pulsatile study night compared with continuous insulin administration, although mean plasma glucose for all subjects was 5.4 ± 0.1 mmol · L^{-1} on the continuous night and 5.2 ± 0.2 mmol · L^{-1} on the pulsatile study night ($P = .2$). In the continuous versus 7-minute study, 4 during the continuous study and 5

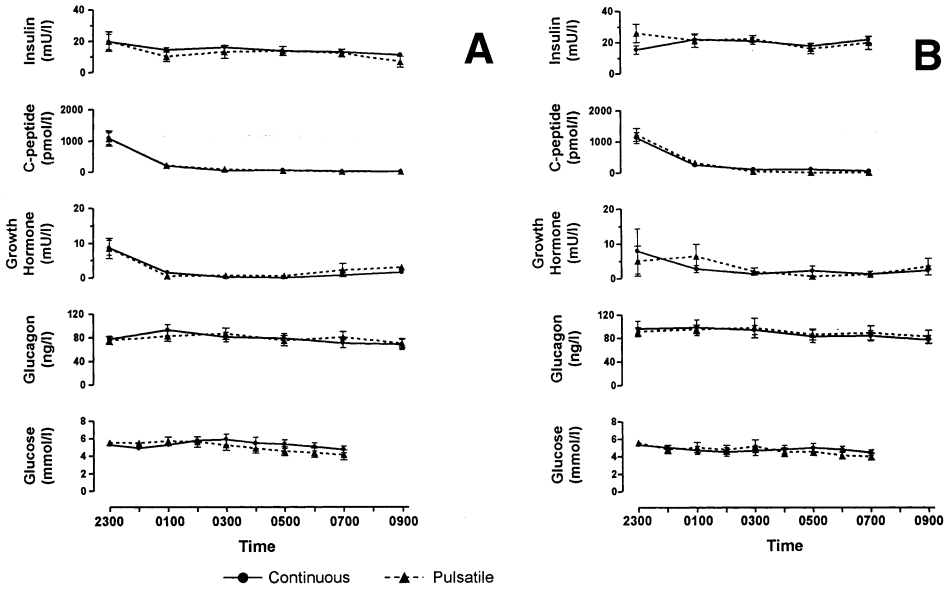

Fig 2. Overnight serum insulin, plasma glucagon, serum growth hormone and plasma glucose levels: (A) continuous *v* 13 minutes; (B) continuous *v* 7 minutes.

subjects during the pulsatile study night required supplemental intravenous dextrose due to hypoglycemia. Mean overnight plasma glucose values were 4.8 ± 0.1 mmol · L^{-1} (continuous) and 4.7 ± 0.2 mmol · L^{-1} (pulsatile) ($P = .5$).

Effective oscillations of insulin were achieved in all patients on the pulsatile infusion study nights (Fig 3), whereas insulin concentration during the continuous infusion study nights were steady at basal concentrations.

Infusion of insulin 1 mU · kg^{-1} · min^{-1} led to comparable steady-state insulin concentrations in the continuous versus 13-minute study (70.5 ± 2.0 mU · L^{-1} *v* 71.7 ± 3.0 mU · L^{-1}; $P = .5$), but despite infusion of similar amounts of insulin differing steady-state insulin concentrations were achieved in the continuous versus 7-minute study (75.9 ± 2.5 mU · L^{-1} *v* 92.2 ± 2.3 mU · L^{-1}; $P = .01$). However, steady-state plasma glucose concentrations during hyperinsulinemia were similar in both studies (continuous *v* 13-minute: 5.0 ± 0.0 mmol · L^{-1} *v* 4.9 ± 0.0 mmol · L^{-1}, $P = .2$; continuous *v* 7-minute: 4.9 ± 0.0 mmol · L^{-1} *v* 5.0 ± 0.0 mmol · L^{-1}, $P = .6$). Exogenous glucose infusion rates required to maintain euglycemia during the final 30 minutes of the glucose clamp (an index of overall insulin sensitivity) did not differ significantly between the pulsatile and continuous study night in either study (continuous *v* 13-minute: 44.3 ± 2.0 μmol · kg^{-1} · min^{-1} *v* 39.3 ± 2.9 μmol · kg^{-1} · min^{-1}, $P = .27$; continuous *v* 7-minute: 34.6 ± 2.5 μmol · kg^{-1} · min^{-1} *v* 41.4 ± 3.2 μmol · kg^{-1} · min^{-1}, $P = .08$). These results are shown in Fig 4.

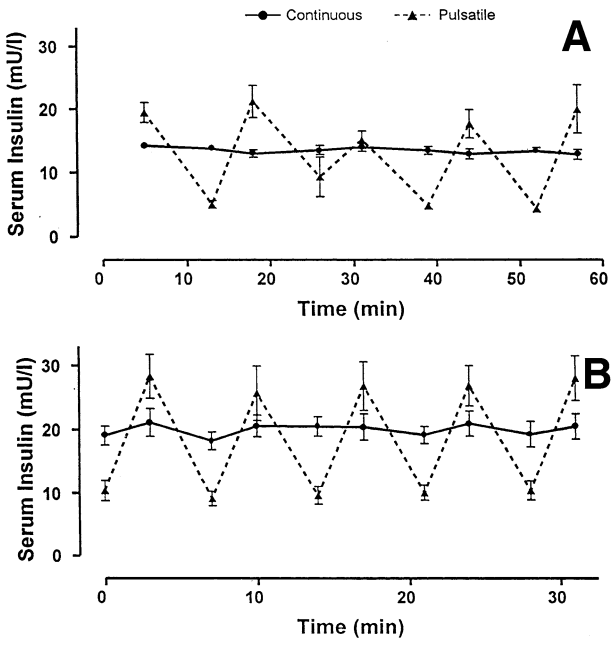

Fig 3. 5:30 to 6:30 AM serum insulin measured before and 3 minutes after each insulin pulse: (A) continuous *v* 13 minutes; (B) continuous *v* 7 minutes.

In the continuous versus 13-minute study, endogenous glucose production both basally (continuous *v* pulsatile: 20.8 ± 2.2 μmol · kg^{-1} · min^{-1} *v* 20.3 ± 1.1 μmol · kg^{-1} · min^{-1}, $P = .81$) and during hyperinsulinemia were similar (continuous *v* pulsatile: 9.8 ± 2.6 μmol · kg^{-1} · min^{-1} *v* 10.5 ± 1.5 μmol · kg^{-1} · min^{-1}; $P = .85$) (Fig 5). Glucose specific activity increased gradually throughout the studies (Fig 5). In the continuous versus 7-minute study, the administration of unlabeled glucose (to prevent hypoglycemia) in some subjects during the basal equilibration period precluded accurate calculation of endogenous glucose production by the Steele equation.

Basal concentrations of serum NEFA, βhydroxybutyrate, and glycerol are shown in Fig 6. NEFA concentrations were similarly suppressed by hyperinsulinemia (continuous *v* 13-minute pulses: 68.3% ± 9.4% *v* 83.0% ± 3.8% reduction, $P = .13$; continuous *v* 7-minute pulses: 66.5% ± 6.8% *v* 65.3% ± 6.9% reduction, $P = .4$). βhydroxybutyrate was similarly reduced in both studies (continuous *v* 13-minute pulses: 36.1% ±

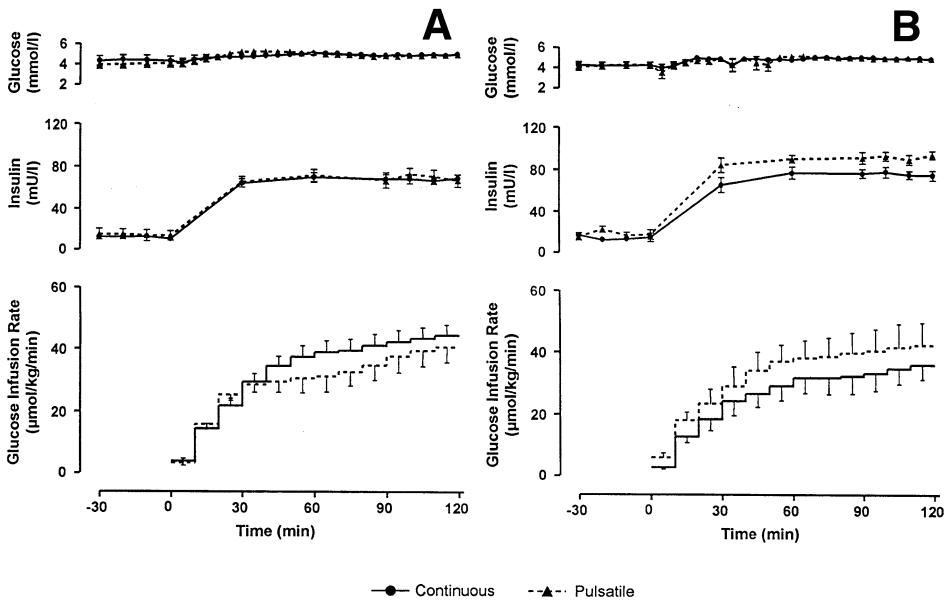

Fig 4. Plasma glucose, serum insulin, and glucose infusion rate (GIR) during hyperinsulinemic clamp: (A) continuous *v* 13 minutes; (B) continuous *v* 7 minutes.

12.3% *v* 50.2% ± 13.1% reduction, $P = .42$; continuous *v* 7-minute pulses: 28.9% ± 19.7% *v* 20.1% ± 17.4% reduction, $P = .8$). Suppression of glycerol was greater on the pulsatile compared with the continuous study night in the continuous versus 13-minute study (continuous *v* pulsatile: 42.5% ± 9.5% *v* 57.9% ± 6.9% reduction, $P = .04$) but was similar in the continuous versus 7-minute study (continuous *v* pulsatile: 59.5% ± 8.6% *v* 70.1% ± 6.9% reduction, $P = .5$).

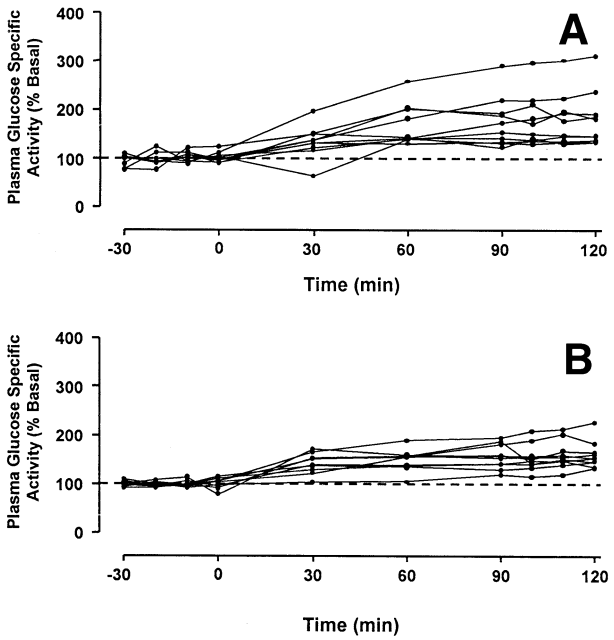

Fig 5. Individual plasma glucose specific activity (% Basal) in continuous *v* 13-minute study: (A) continuous study night; (B) pulsatile study night.

DISCUSSION

We previously have reported a negative correlation between endogenous insulin pulse frequency and peripheral insulin action (as represented by glucose clearance) in both normal controls and non-obese type 2 diabetic subjects.[7,8] The mechanism underlying this relationship was unclear, but we hypothesized that basal insulin pulses may have a priming effect on peripheral insulin sensitivity that is revealed on subsequent challenge with hyperinsulinemia. No previous study has investigated this potential physiological mechanism and for this reason our current study was devised. Insulin was administered overnight at basal levels— either by continuous infusion or in pulses at 13- or 7-minute frequency. This was followed the next morning by a hyperinsulinemic clamp to assess any potential priming effect of pulsatile insulin.

The pattern of a 2-minute pulse followed by an 11-minute interval was based on early reports of insulin pulse frequency in normal man[2] and replicated the protocols used by Matthews et al[4] and later demonstrated by Paolisso et al[6] to be effective in reducing plasma glucose compared with pulses at lower frequency. More recently, portal vein sampling techniques and in vitro assessment of beta-cell secretion have suggested a higher endogenous frequency of pulsatile insulin release at 7.5 to 9.0 pulses per hour[9,15] and therefore the 7-minute pulse frequency study was devised.

An enhanced hypoglycemic effect after 7 hours of overnight pulsed insulin compared with continuous administration at basal levels has previously been reported by Matthews et al.[4] In the continuous versus 13-minute study, 4 of 10 subjects did have an enhanced hypoglycemic effect overnight with pulsatile insulin, although we did not demonstrate any overall difference in overnight plasma glucose concentrations. In the continuous versus 7-minute study no individual or overall differences were seen, although the administration of intravenous glucose in several subjects may have masked any potential difference.

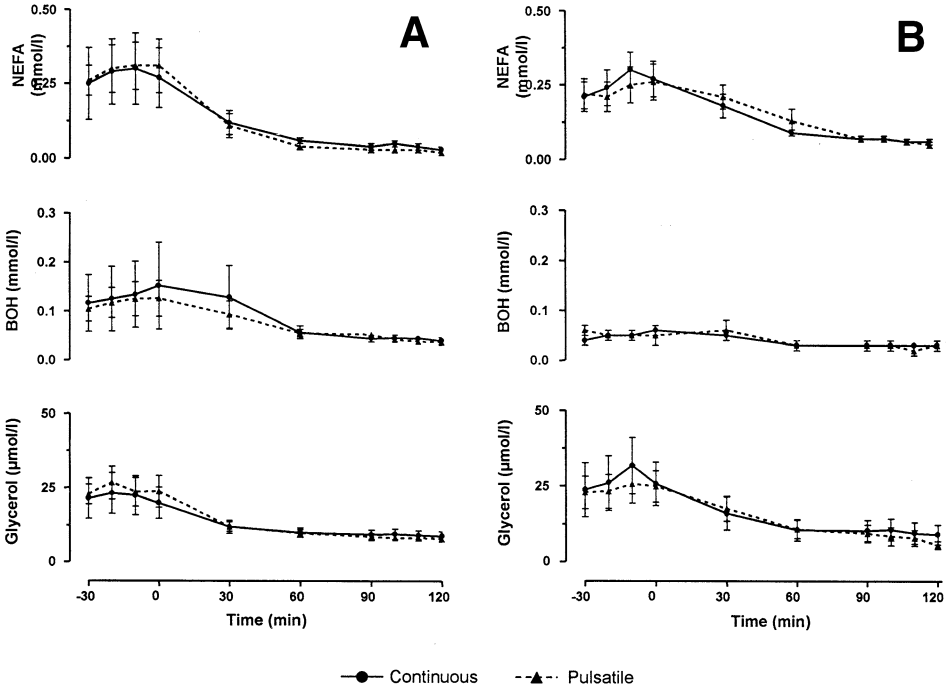

Fig 6. Serum NEFA, βhydroxybutyrate, and glycerol during hyperinsulinemic clamp: (A) continuous *v* 13 minutes; (B) continuous *v* 7 minutes.

Additionally, glucose concentrations are critically influenced by glucagon. In the study by Matthews et al[4] glucagon was replaced at 12.5 $\mu g \cdot h^{-1}$ (with plasma levels not reported), while the replacement dose of 0.03 $\mu g \cdot kg^{-1}\ h^{-1}$ that we employed was considerably lower. We achieved similar mean plasma glucagon concentrations (76.8 $pg \cdot mL^{-1}$) to those of Paolisso et al,[16] who reported that the greater efficacy of pulsatile insulin was lost if glucagon replacement was excessive and described optimal plasma glucagon concentrations around 75 $pg \cdot mL^{-1}$. In addition, while glucagon is also normally secreted in a pulsatile fashion, we replaced it by continuous infusion.[17] It is possible therefore that differing glucagon replacement may have altered either the hepatic or peripheral response to overnight insulin thus accounting for the lack of difference we noted in overnight glucose concentrations.

A possible reason for our failure to demonstrate enhancement of insulin action with pulsatile administration may have been the development of a counterregulatory response to overnight hypoglycemia. During the continuous versus 13-minute study, 4 subjects had a venous plasma glucose approaching 3.5 $mmol \cdot L^{-1}$ after 6 hours of pulsatile insulin, with 2 subjects exhibiting a similar level of hypoglycemia during the continuous study night. In the continuous versus 7-minute study, 5 subjects with pulsatile insulin administration and 4 with continuous insulin were given supplemental intravenous glucose for the development of plasma glucose less than 3.5 $mmol \cdot L^{-1}$. The threshold for an epinephrine response to hypoglycemia is an arterialized venous plasma level of around 3.5 $mmol \cdot L^{-1}$,[18] and while we did not measure plasma catecholamines it is possible that these subjects may have counter-regulated. It is recognized that a counter-regulatory response will both increase hepatic glucose output and reduce peripheral insulin action and therefore any potential improvement in insulin action with pulsatile administration may have been masked.[19,20] Another possible counter-regulatory hormone, cortisol, was measured throughout the night but levels did not differ between study protocols (data not shown). The administration of intravenous glucose in the continuous versus 7-minute study may additionally have masked any potential difference.

Another possible reason for the lack of enhanced effect on insulin action of pulsatile insulin may be that a longer duration of pulses is required before a significant difference can be demonstrated. Pulsatile insulin was administered in our study for 10 hours prior to hyperinsulinemia as it was felt that longer study duration would not be acceptable to our subjects.

Mean basal endogenous glucose production values after overnight infusion in the continuous versus 13-minute study were 20.3 ± 1.1 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (pulsatile) and 20.8 ± 2.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (continuous). These levels are somewhat higher than those reported previously for normal individuals and may represent the effect of counter-regulation as discussed above. Suppression of endogenous insulin release by somatostatin will result in portal hypoinsulinemia thus also potentially increasing hepatic glucose production. Furthermore, the necessity in human studies to replace insulin and glucagon peripherally obviously differs from their normal portal release, a factor that may additionally have altered hepatic glucose metabolism.

In the continuous versus 7-minute study, despite identical insulin infusion rates during hyperinsulinemia, steady-state insulin levels were higher during the pulsatile study than the continuous (pulsatile 92.2 ± 2.3 $mU \cdot L^{-1}$ *v* continuous 75.9 ±

2.5, $P = .01$) during the final 30 minutes of the clamp. This might explain the observation that the glucose infusion rate was slightly but not significantly higher following pulsatile infusion. However, insulin action, expressed in terms of the insulin sensitivity index (glucose infusion rate/insulin concentration) indicates no difference between the pulsatile and continuous study nights (pulsatile 0.45 ± 0.06 *v* continuous 0.53 ± 0.07, $P = .2$). The reason for the differing clamp insulin values is unclear. Suppression of endogenous insulin secretion was complete and therefore similar in both the pulsatile and continuous studies. Assays were completed in batches with equivalent number of pulsatile and continuous study samples and the insulin assay coefficient of variation was less than 5% making assay variability unlikely. It is possible that differences in insulin clearance could explain the higher clamp insulin levels in the pulsatile study clamp.

Defects in both insulin secretion and insulin action are present in several conditions associated with the metabolic syndrome and it is unclear whether they are simply related epiphenomena or have a cause and effect relationship. If a causal relationship exists, then which defect is primary is of interest with major implications for our understanding of the pathophysiology of insulin-resistant states. Our data are consistent with either a priming effect on insulin action of pulsatile insulin that was masked by methodological difficulties in the current study or that abnormalities of insulin secretory pulses may not be important in the development of peripheral insulin resistance.

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