Similar Metabolic Effects of Pulsatile Versus Continuous Human Insulin Delivery During Euglycemic, Hyperinsulinemic Glucose Clamp in Normal Man

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SUMMARY

Seven normal volunteers were studied on two different occasions during which 4-h pulsatile (PULS: 0.8 mU · kg^{-1} · min^{-1}, 7.5 min of 15) and continuous (CONT: 0.4 mU · kg^{-1} · min^{-1}) intravenous (i.v.) infusions of human insulin (Actrapid HM, Novo) were randomly compared. A euglycemic glucose clamp was performed and a 3-^{3}H-glucose infusion was used for determination of endogenous glucose production (EGP) and metabolic clearance rate (MCR) of glucose. Plasma glucose was similar in both conditions; plasma insulin was stable at about 29 mU/L (CONT) and fluctuated between 10 and 45 mU/L (mean: 28, PULS). Exogenous glucose infused was 1.137 ± 0.058 and 1.088 ± 0.099 g · kg^{-1} · 4 h^{-1} in CONT and PULS, respectively (NS). EGP was totally suppressed in both conditions. Glucose MCR increased similarly to a maximum of 6.71 ± 0.19 (CONT) and 6.79 ± 0.59 (PULS) ml · kg^{-1} · min^{-1} during the fourth hour. C-peptide plasma levels remained stable, whereas plasma glucagon, free fatty acids, and 3-hydroxybutyrate were similarly suppressed in both tests. Thus, under these conditions, pulsatile and continuous insulin infusions have similar metabolic effects. These data contrast with those of Matthews et al. (1983) who reported that, at lower plasma concentrations (5–19 mU/L), pulsatile insulin had greater hypoglycemic effect than did continuous delivery. It is concluded that pulsatile insulin shows no greater activity under normoglycemic, moderately hyperinsulinemic conditions in man. DIABETES 1984; 33:1169–74.

In 1977, Goodner et al.[1] demonstrated the existence of synchronous and sustained oscillations in plasma concentrations of insulin, glucagon, and glucose in fasting monkeys. Soon thereafter, similar cyclic oscillations of glucose and insulin were shown to be present in humans as well.[2] In man, the basal plasma insulin concentration cycled regularly with a mean period of 13 min and a mean amplitude of 1.6 mU/L. A concurrent plasma glucose cycle, 2 min in advance of the insulin cycle, was also demonstrated.[2] Subsequent reports have focused on the origin and the control of these oscillations. They suggested that the oscillatory stimulus arises in the pancreas itself,[3] and showed that the pulsation frequency was stable through various neural blockades[4,5] or small perturbations with exogenous glucose or insulin,[5] while stimulation of insulin secretion by intravenous (i.v.) glucose, tolbutamide, or sodium salicylate increased the amplitude of the insulin oscillations, the frequency remaining stable.[5] These stable oscillations were found to be replaced by brief, irregular oscillations in type II diabetic subjects and this abnormal rhythm was postulated as a contributing factor to the basal hyperglycemia that occurs in these patients.[6]

Recently, pulsatile delivery of insulin was shown to have a greater hypoglycemic effect than continuous delivery during an overnight infusion in normal subjects infused with somatostatin to suppress their basal endogenous insulin secretion.[7] We were interested in knowing whether pulsatile insulin retained its enhanced metabolic effect when infused at rates permitting the achievement of plasma insulin levels similar to those observed in the postprandial state, in which case the addition of pulsations to the delivery system of the insulin pumps, currently in use in diabetic patients, might prove to be of help in reducing the hyperinsulinism observed in these patients and its putative effect on the acceleration of atherosclerosis.

MATERIALS AND METHODS

Subjects. Informed consent was obtained from 7 healthy, male, adult volunteers, aged 25 ± 3 yr (range 20–29 yr). All were within 10% of their ideal body weight (Metropolitan Life Insurance Tables) and none had a family history of diabetes

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mellitus. All were consuming a regular, weight-maintaining diet and none were taking any medications.

Experimental protocol. After an overnight fast (9–11 h), the subjects were admitted to the Metabolic Unit of the University Hospital between 7:00 and 7:30 a.m. They were placed at bed rest and maintained supine throughout the whole experiment. Three 18-gauge polyethylene catheters were inserted i.v. into each subject. One catheter, in an antecubital vein, was used for intermittent blood sampling. A second, distally on the same arm, consisted of a double-lumen catheter for continuous blood withdrawal by a Biostator (Life Science Instruments, Miles Laboratories, Elkhart, Indiana). The third catheter, inserted in a large vein in the other arm, was used for all infusions. In all experiments, the rates of glucose production and utilization were determined using a primed (25 μCi)-continuous (0.25 μCi · min^{-1}, 2 μCi · ml^{-1}) infusion of 3-^{3}H-glucose (New England Nuclear, Boston, Massachusetts; sp act 11.5 Ci · $mmol^{-1}$) dissolved in saline. A 2-h equilibration period was allowed before initiation of the experiments, during which the Biostator was calibrated and the different solutions prepared. Each subject was tested on two occasions, in random order, separated by at least 1 wk. On one day, a 4-h continuous i.v. infusion of semisynthetic human insulin (Actrapid HM, Novo Industri, Copenhagen, Denmark) at a rate of 0.4 mU · kg^{-1} · min^{-1} was performed. On the other day, an identical amount of insulin in toto was given using pulses of 7.5-min duration (during which insulin was infused at a rate of 0.8 mU · kg^{-1} · min^{-1}) followed by gaps of 7.5 min during which no insulin was infused. The accessory channel of the Biostator infusion pump was used to infuse the insulin and was manually switched during the intermittent infusion.

Along with the insulin infusion, variable amounts of glucose were infused, following the euglycemic glucose clamp method initially described by DeFronzo et al.,[8] to maintain the blood glucose at approximately the same concentration that had been present in the basal state. The rate of the glucose infusion was automatically adjusted, using the Biostator algorithms and the following constants: KR = 165, KF = 45, QD = 25, and RD = 250.

Insulin was dissolved in 0.9% NaCl containing 0.6% human serum albumin (Institut Mérieux, Lyon, France). Glucose was infused as a 50% solution to which 0.26 meq KCl/ml was added to prevent hypokalemia. The initial serum potassium concentration was 4.5 ± 0.7 meq/L, and averaged 3.8 ± 0.2 meq/L after the 4-h insulin infusion. The 3-^{3}H-glucose was infused by a separate high-precision pump (PS 2000, Robert et Carrière, Antony, France). Blood samples were obtained at 7.5-min intervals throughout the experimental protocol except for during the third hour, when sam-

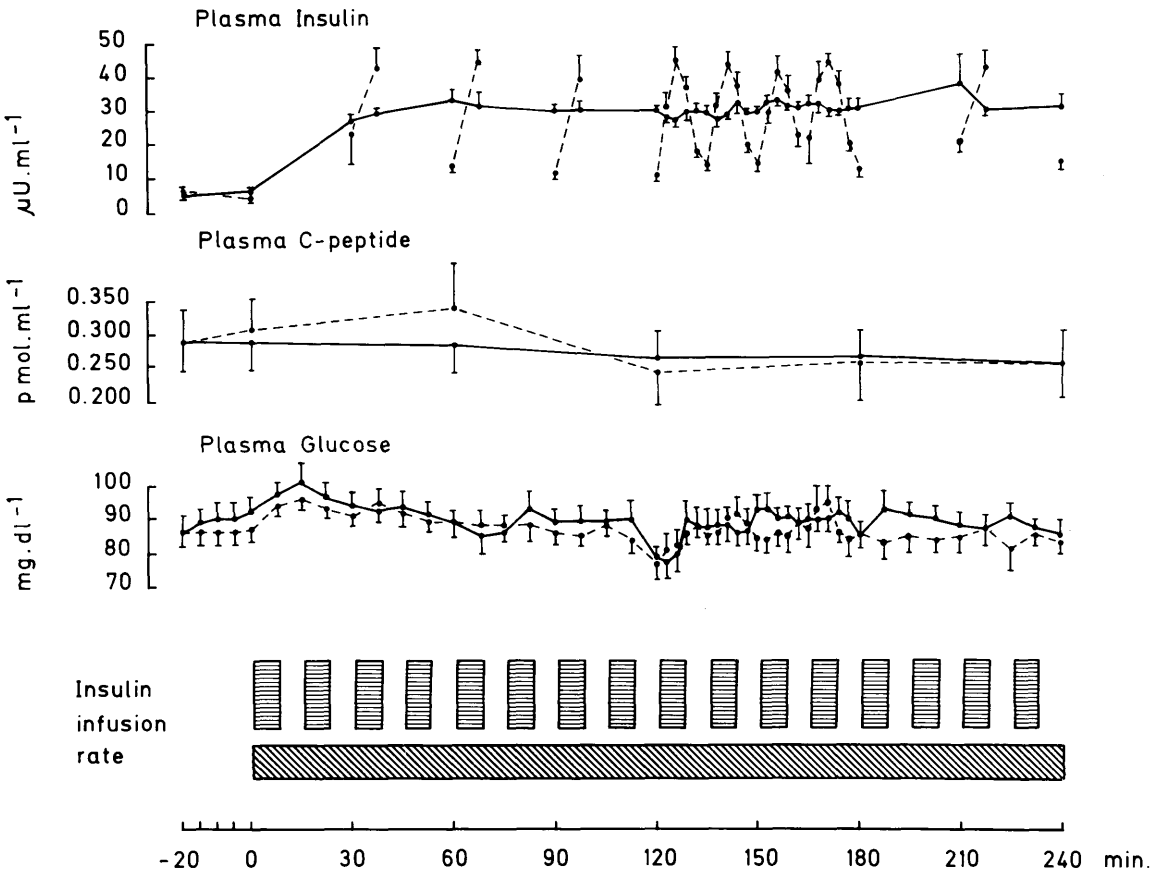

FIGURE 1. Plasma glucose, insulin, and C-peptide during continuous (●——●) or pulsatile (●----●) insulin infusion. Multiple collections of blood permitting the demonstration of oscillations in plasma insulin were performed during the third hour of the test only. Insulin infusion rates during the two protocols are indicated schematically at the bottom of the graph; in the pulsatile mode of administration, insulin was infused at a rate of 0.8 mU · kg^{-1} · min^{-1} during periods of 7.5 min followed by gaps of 7.5 min during which no insulin was infused; in the continuous mode of administration, insulin was infused at a constant rate of 0.4 mU · kg^{-1} · min^{-1}. Results are expressed as mean ± SEM (N = 7).

ples were obtained every 3 min. Hourly samples for C-peptide, glucagon, 3-hydroxybutyrate, and FFA were collected in chilled 10-ml tubes containing 1 ml of an EDTA-Trasylol solution (Trasylol, Bayer 5000 U/ml and Na_2EDTA 1.2 mg/ml). Blood for insulin determination was collected in chilled, heparinized tubes at various intervals of time that are indicated in the graphs. Blood for plasma glucose and glucose specific activity was collected in chilled tubes containing a trace of sodium fluoride.

Analytic procedures. All blood samples for hormone and metabolite determinations were centrifuged immediately after each experiment and the plasma stored at −20°C until assay except for plasma glucose, which was determined immediately after the experiment using the hexokinase method adapted to the Technicon AutoAnalyzer.[9]

Plasma 3-^{3}H-glucose specific activity was determined as follows: 1-ml aliquots of plasma were deproteinized according to Somogyi.[10] The resultant filtrate was aliquoted in two 0.5-ml samples that were lyophilized to remove the tritiated water resulting from the metabolization of 3-^{3}H-glucose during the experiment. The dry residue was resuspended with 0.5 ml of distilled water and its radioactivity counted in a refrigerated liquid scintillation counter after addition of 5 ml of Aquasol (New England Nuclear). The average glucose radioactivity of each plasma sample was divided by its glucose concentration to obtain the glucose specific activity. Similarly, four aliquots of the infused solution of 3-^{3}H-glucose were counted and the average radioactivity, as well as the infusion rate (verified by measuring the volume of the 3-^{3}H-glucose infusate before and after each experiment) were used in subsequent calculations.

Plasma insulin was measured according to the C-method of Hales and Randle[11] using centrifugation instead of filtration, plasma free fatty acids (FFA) according to Dole and Meinertz,[12] and plasma glucagon according to Luyckx[13] using Unger's 30K antiserum and ^{125}I-glucagon obtained from New England Nuclear. C-peptide was determined by the method of Heding,[14] and 3-hydroxybutyrate by a spectrophotometric enzymatic[15] method using reagents obtained from Boehringer (Mannheim, FRG). The plasma samples obtained during the two experiments in each subject were assayed within the same assay series to eliminate the effect of interassay variations.

Calculations. The amount of glucose infused necessary to maintain the basal glycemia during the insulin infusion (glucose infusion rate, GIR) was calculated for 30-min intervals throughout all experiments and expressed in milligrams per minute and per kilogram of body weight. Glucose production in the basal state was determined by dividing the 3-^{3}H-glucose infusion rate (count/min) by the steady-state plateau of 3-^{3}H-glucose specific activity (five measurements) achieved during the last 20 min before the insulin infusion was started. In basal conditions, the rate of glucose appearance (R_a) is equal to the rate of glucose disappearance (R_d), and R_a represents the basal hepatic glucose production since no significant renal glucose production occurs after an overnight fast in normal subjects. The basal metabolic clearance rate of glucose was calculated by dividing the basal R_d by the mean plasma glucose during the control period. During the insulin infusion, a non-steady-state condition in glucose specific activity exists and Steele's equations in their derivative form[16] with a value of 0.65 as the pool fraction[17] were used to calculate the R_a, R_d, and MCR. In these conditions, the R_a measures the total amount of glucose appearing in the plasma and, during a glucose clamp, represents the sum of the glucose infused and the endogenous hepatic glucose production. The hepatic glucose production is easily calculated by subtracting the glucose infusion rate (GIR) from the rate of glucose appearance (R_a). These calculations were performed with a program written in Basic on an Apple II desk computer, and the integrated values for 30-min periods of R_a, R_d, and MCR are presented in the text and graphs.

All data are presented as mean ± SEM. All statistical comparisons between the two modalities of insulin infusions were calculated by paired *t*-test analysis, and a P-value <0.05 was considered of statistical significance.

RESULTS

Plasma glucose, insulin, and C-peptide concentrations (Figure 1). Plasma glucose levels during the continuous insulin infusion (89.6 ± 0.5 mg · dl^{-1}) and pulsatile insulin infusion (87.1 ± 0.6 mg · dl^{-1}) were similar and did not differ significantly from basal plasma glucose concentrations (89.4 ± 1.0 and 86.2 ± 0.2 mg · dl^{-1}, respectively). The coefficient of variation of blood glucose values in each subject for the period of the clamp was 8.4 ± 0.9%. Basal plasma insulin levels were similar before the continuous and pulsatile insulin infusions (5.8 ± 0.4 and 5.4 ± 0.6 μU · ml^{-1}, respectively). During the continuous infusion, a stable plateau at ≈30 μU · ml^{-1} was rapidly attained. During the third hour of the continuous infusion, plasma samples were obtained every 3 min; the average plasma insulin was 29.3 ± 0.3 μU · ml^{-1} with a coefficient of variation of 5.4%. During the third hour of the pulsatile infusion, the mean plasma insulin (27.8 ± 2.5 μU · ml^{-1}) was not statistically different from that of the continuous infusion, but exhibited large, regular oscillations with a mean amplitude of 29 ± 3 μU · ml^{-1} (14–43 μU · ml^{-1}) and a period of 15 min, as reflected by a coefficient of variation of 42%. The areas under both insulin curves were similar (1593 ± 76 and 1623 ± 144 μU · ml^{-1} · min for continuous and pulsatile delivery, respectively), reflecting the fact that indeed similar amounts of insulin had been infused in the two protocols. The endogenous insulin secretion, assessed by circulating C-peptide determinations, remained unchanged during the two tests and no significant differences were noted between the continuous and pulsatile infusions.

Glucose infusion rate (GIR), R_a, R_d, and MCR (Figure 2). Similar total amounts of glucose were infused by the Biostator in response to the continuous and pulsatile insulin infusion: 1.137 ± 0.058 and 1.088 ± 0.099 g · kg^{-1} · 4 h^{-1}, respectively (NS). The two curves follow a similar pattern, rising progressively during the first 2 h of the infusion to reach a plateau at approximately 5.7 mg · kg^{-1} · min^{-1} during the last 2 h. The endogenous glucose production, as assessed by the difference between the R_a and the GIR, was similar before the continuous and pulsatile insulin infusions: 2.23 ± 0.07 and 2.22 ± 0.09 mg · kg^{-1} · min^{-1}, respectively. It was similarly affected by both insulin infusions, decreasing progressively during the first 60 min to be totally suppressed from then until the end of the infusions. The glucose utili-

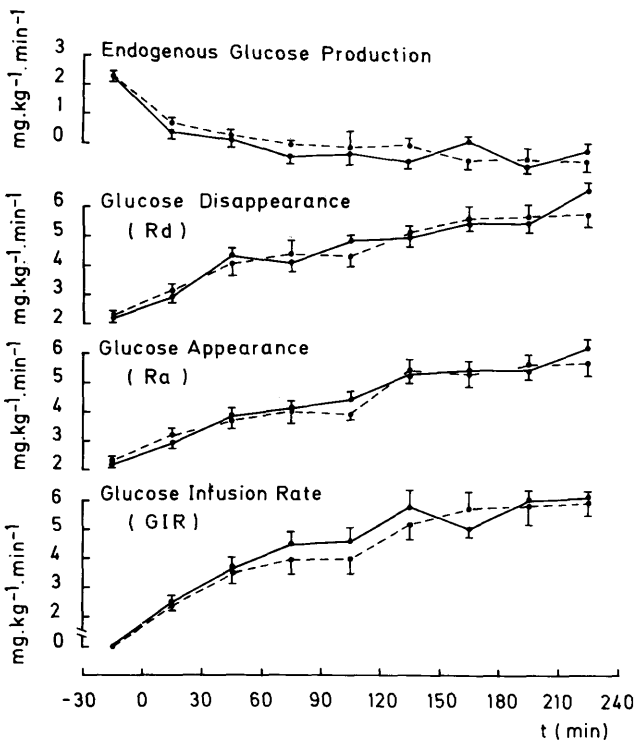

FIGURE 2. Comparison of the effects of continuous (●——●) and pulsatile (●----●) insulin infusion on the various parameters of glucose metabolism during euglycemic, hyperinsulinemic glucose clamp. GIR: glucose infusion rate by the Biostator. R_a (total amount of glucose appearing in the plasma) and R_d (rate of glucose disappearance) were derived from changes in 3-^{3}H-glucose specific activity. Endogenous glucose production corresponds to R_a − GIR. Results are expressed as mean ± SEM (N = 7).

zation, measured by the R_d of glucose, equal by definition to the R_a of glucose in basal conditions, responded to the continuous and pulsatile insulin infusions by a similar and progressive rise to a maximum of 5.96 ± 0.25 and 5.61 ± 0.38 mg · kg^{-1} · min^{-1}, respectively, during the last hour of the infusions (NS). The MCR of glucose was 2.53 ± 0.06 and 2.59 ± 0.06 ml · kg^{-1} · min^{-1} before the continuous and pulsatile insulin infusions, respectively (NS); it responded similarly to both insulin infusions by a progressive rise to reach a maximum of 6.71 ± 0.19 and 6.79 ± 0.59 ml · kg^{-1} · min^{-1} during the last hour of the continuous and pulsatile infusions, respectively (NS).

Glucagon, free fatty acids (FFA), and 3-hydroxybutyrate (Figure 3). Plasma glucagon, FFA, and 3-hydroxybutyrate levels were similar in basal conditions before the continuous and pulsatile infusions and, as shown in Figure 3, similarly suppressed in both conditions. None of the minor differences observed attained the level of statistical significance.

DISCUSSION

Insulin is secreted in a pulsatile manner by the pancreas,[1-5] as is arginine vasopressin by the pituitary[18] and LHRH by the hypothalamus.[19] The pulsatile secretion is present in basal conditions as well as when insulin secretion is stimulated by an i.v. glucose infusion, in which circumstances the pulsations are of greater amplitude but retain the same period.[5] In type II diabetic subjects, small, irregular oscillations (7-min period) are superimposed on more regular oscillations with a mean period of 40 min[6] in contrast to the relatively stable 14-min period rhythm present in normal subjects.[5] This abnormal pattern of insulin secretion has been incriminated as one of the potential factors responsible for the development of glucose intolerance in these patients.[6]

Type I diabetic subjects are totally dependent on the supply of exogenous insulin, which is not currently delivered in a pulsatile way. Whether these oscillations are of any metabolic, and consequently clinical, importance is a question recently addressed by Matthews et al.[7] and the present study. The study of Matthews et al.[7] demonstrated a greater hypoglycemic effect of pulsatile insulin, when compared with a continuous infusion, at plasma insulin levels similar to those observed in the postabsorptive state. The present study, conducted at higher insulin levels (similar to those present in the postprandial state), failed to demonstrate any difference in several metabolic parameters and substrates directly influenced by insulin.

It is now considered that the insulin receptor affinity, as well as the number of insulin receptors, are directly influenced by the ambient insulin concentration at the receptor

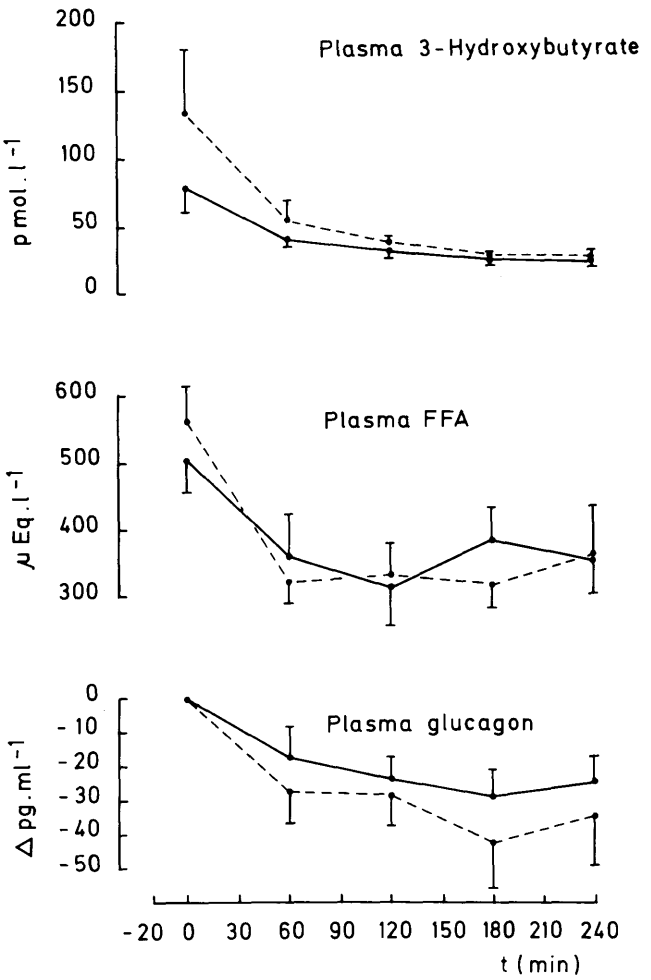

FIGURE 3. Changes in plasma 3-hydroxybutyrate, free fatty acids (FFA), and glucagon during continuous (●——●) or pulsatile (●----●) insulin infusion. Results are expressed as mean absolute values (± SEM, N = 7) for 3-hydroxybutyrate and FFA and as mean changes from basal level (Δ) for plasma glucagon.

site (review in ref. 20). Matthews et al. observed that the enhanced metabolic activity of pulsatile insulin was accompanied by a greater binding affinity of monocyte insulin receptors after the pulsatile infusion than after the continuous infusion; therefore, they postulated that pulsatile insulin, with its rapid alternance of high and low insulin concentrations and the consequent diminished exposure time to high insulin, did not allow for the downregulation mechanism mentioned above to occur.[7]

However, one of the prerequisites for this theory to be valid is that the insulin receptor itself actually "sees" the pulsation; that is, the measured variations in insulin plasma levels must be present at the receptor site in the various tissues as well. If one accepts a three-compartment model to describe the kinetics of insulin,[21] the first compartment represents the plasma, wherein a molecule of insulin stays an average of 2.3 min. The mean time of stay in the second compartment is 2.5 min, and this compartment is considered to represent highly vascularized tissues, predominantly the liver. The last compartment represents the muscles and adipose tissue, wherein the mean time of stay for an insulin molecule has been calculated to be about 50 min.[21] From these data, one can conclude that the liver must be the most affected by the insulin pulsations, since the mean time of stay for an insulin molecule in that compartment is rather small (2.5 min) when compared with the 14-min period of the pulsation. Moreover, the liver, because of its exposure to portal blood, is exposed to pulsations of higher amplitude than the other organs. In contrast, the insulin pulsations are probably less effective or ineffective at the level of the peripheral tissues (muscles, adipose tissue), since the mean time of stay of an insulin molecule in that compartment is long (50 min) when compared with the period of the pulsation.

At low insulin levels, such as in the postabsorptive state, small increments in plasma insulin levels will have a major effect on the hepatic glucose output and little or no effect at the periphery.[22] At higher insulin levels, such as those attained in the present study, the hepatic glucose output is almost completely suppressed by the insulin infusion, and increments in plasma insulin levels or efficacy will result in an increase in the peripheral consumption of glucose.[22]

In light of these data, one can try to understand the apparently contradictory results of this study and the one reported by Matthews et al.[7] Their study was conducted at lower plasma insulin levels (5–19 $\mu U \cdot ml^{-1}$) and the increased hypoglycemic activity of pulsatile insulin was probably secondary to a more effective suppression of the hepatic glucose output. However, that parameter was not measured and this is only speculative. In contrast, our study was conducted at insulin levels sufficient to completely suppress hepatic glucose output. Consequently, for the pulsatile infusion to be superior to the continuous infusion, it should have resulted in an increased glucose utilization by the peripheral tissues. Under hyperinsulinemic, euglycemic conditions, the increased utilization of glucose is accounted for by the muscles (85%),[23] brain (8%, insulin independent?), splanchnic bed including the liver (6%),[24] and adipose tissue (1%).[25] Moreover, under the same conditions, although insulin effectively inhibits hepatic glucose production, its ability to stimulate net hepatic glucose uptake is negligible.[24] In the light of these data, we conclude that the muscles play the predominant role in glucose utilization and, as discussed above, may be less affected by the pulsations of insulin. Other differences in the experimental protocols may also contribute to explain the discrepancy between the results of this study and those reported by Matthews et al.[7] Indeed, it has recently been shown by Ciaraldi and Olefsky[26] that the rate of decay of insulin effect is critically dependent on pulse duration; therefore, the 7.5-min pulses might have been long enough to obscure any benefit due to pulsatile administration. Second, the present study lasted only 4 h, whereas it took 7 h to demonstrate a greater hypoglycemic activity of pulsatile insulin in the protocol of Matthews et al.[7] However, hyperinsulinemic conditions, such as in this study, rarely last more than 2 h under normal conditions (in contrast to the prolonged hyperinsulinemia often seen in obese, type II diabetic subjects) and, therefore, a 4-h study seemed a reasonable endpoint in young healthy subjects. Third, in the present study, a hyperinsulinemic clamp was imposed on fasting subjects with basal plasma glucose averaging 87–90 $mg \cdot dl^{-1}$; these conditions clearly do not truly mimic the postprandial state, in which plasma glucose levels of 120–140 $mg \cdot dl^{-1}$ are seen.

We cannot exclude the possibility that, at higher glucose levels, a difference between the two modes of insulin administration may have developed. Finally, in their study, Matthews et al.[7] used somatostatin to suppress pancreatic insulin output. One can speculate that the somatostatin-induced portal hypoinsulinemia might have rendered the liver even more sensitive to the fluctuations in insulin levels.

We conclude that pulsatile insulin, even though more effective than continuous insulin under near-to-basal conditions (5–19 $\mu U \cdot ml^{-1}$) as demonstrated by Matthews et al.,[7] shows no greater activity under normoglycemic, moderately hyperinsulinemic (14–43 $\mu U \cdot ml^{-1}$) conditions.

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