Sleep-Associated Fall in Glucose Disposal and Hepatic Glucose Output in Normal Humans

Putative Signaling Mechanism Linking Peripheral and Hepatic Events

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Values reported for basal hepatic glucose production and glucose utilization do not reflect metabolic changes occurring during sleep. To determine the effect of sleep with its associated lowered metabolic rate and thermogenesis on glucose kinetics and gluconeogenic substrate availability, 11 normal volunteers underwent an overnight study in which [3-^{3}H]glucose was infused. Despite decreased insulin secretion, a fall in hepatic glucose output was observed with sleep that was synchronous with a reduction in glucose utilization and lipolysis (decreased plasma glycerol and free fatty acids). When activity was increased, these parameters rose toward previously reported basal levels. Prevention of sleep in 6 additional subjects attenuated the fall in glucose utilization and production as well as the fall in glycerol and free fatty acids despite similar insulin and counterregulatory hormone profiles. We suggest that sleep-associated metabolic changes produce a peripheral signal(s) that modulates hepatic glucose production in humans. *Diabetes* 38:285–90, 1989

During the postabsorptive period, normal plasma glucose concentrations are maintained by an exquisitely controlled balance between glucose utilization and hepatic glucose production (1,2). Current isotopic techniques for the measurement of glucose kinetics in vivo have resulted in a vast literature exploring the metabolism of glucose at rest (3) and during exercise (4) in normal and diabetic humans. However, the effects of sleep on glucose kinetics have been largely inferred. Based on the fall in metabolic rate known to occur with sleep, changes in the rate of carbohydrate flux are also likely (5).

Glucose utilization would be expected to fall during sleep as a result of decreased muscular activity. Because plasma glucose levels remain unchanged or even fall slightly overnight, a fall in glucose utilization would necessarily be accompanied by a fall in hepatic glucose output (HGO). The purpose of our study was to examine glucose kinetics (glucose utilization and HGO) during sleep in normal humans and to seek a peripheral signaling mechanism that might explain the changes in HGO in response to decreased peripheral glucose utilization.

Glucose 1 mM = 18 mg/dl	Insulin 1 pM = 0.139 μU/ml

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MATERIALS AND METHODS

Seventeen normal volunteers [mean ± SE age 25 ± 1 yr, body mass index (BMI) 24.1 ± 0.6 kg/m^2] were admitted to the Clinical Research Center at the Medical College of Virginia at 1800 for the overnight studies. Twelve of the subjects were male (BMI 24.9 ± 1.1 kg/m^2), and 5 were female (BMI 23.5 ± 0.9 kg/m^2) in the midfollicular phase of their menstrual cycle. None of the subjects were trained athletes. After a meal at 1800 consisting of ~55% carbohydrate, 15% protein, and 30% fat, the subjects were fasted until completion of the study at 0800 the next morning. Eleven of the subjects were permitted to sleep from 2430 until 0700, when they were awakened for the final 60 min of the study. To compare the effect of sleep to that of the postabsorptive period, 6 additional subjects were studied while awake during the same period. These subjects were permitted to watch television or read while at bedrest and were regularly engaged in conversation to maintain alertness. Core temperature was recorded at 30-min intervals with a rectal temperature probe. Heart rate was recorded continuously with a standard electrocardiographic monitor (Honeywell).

In all subjects, a primed (25-μCi) continuous (0.25-μCi/min) infusion of D-[3-^{3}H]glucose (New England Nuclear, Boston, MA) was administered via a forearm vein from 2200 until completion of the study for the determination of glucose specific activity. An indwelling venous catheter was inserted

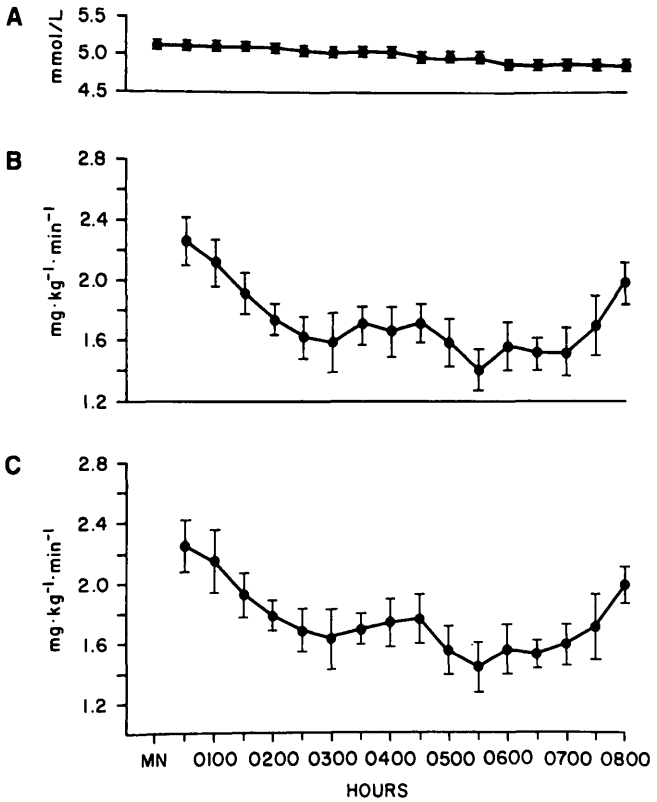

FIG. 1. Plasma glucose (*A*) and rates of glucose appearance (*B*) and disappearance (*C*) during overnight study in normal volunteers. Subjects were allowed to sleep from 2430 to 0700. Results are expressed as means ± SE; *n* = 11.

in the contralateral forearm and was kept patent with an infusion of 0.9% NaCl. No heparin was used in the studies. Blood samples were obtained at 30-min intervals from 2400 to 0800 for determination of glucose specific activity and measurement of plasma hormones, lactate, alanine, glycerol, and free fatty acids (FFAs). In preliminary studies (n = 4), simultaneous blood samples were obtained from indwelling radial arterial and brachial venous catheters. All subsequent analyses were performed on venous samples only.

Plasma glucose was measured by the glucose oxidase method (Beckman, Fullerton, CA). Plasma insulin (6), C-peptide (7), growth hormone (8), and glucagon (9) were determined by double-antibody radioimmunoassays. Glucagon determinations were performed in the laboratory of R. Unger (University of Texas Health Science Center, Dallas). Cortisol was measured by direct radioimmunoassay (γ-coat assay, Clinical Assays, Cambridge, MA). Plasma FFA was determined by enzymatic methods (10). Blood samples for determination of glucose specific activity and measurement of lactate, alanine, and glycerol were immediately deproteinized with 3 M ice-cold perchloric acid. The supernatant was neutralized with 3M KOH, and the resulting supernatant was assayed for L-lactate (11) and glycerol (12). After isotopic equilibration (2 h), rates of glucose appearance (R_a) and disappearance (R_d) were calculated from Steele's equations as modified by DeBodo et al. (13) with a glucose-pool fraction of 0.65 (14). In the absence of exogenous glucose administration during the overnight fast, the glucose R_a may be equated with HGO. Comparison of the two groups was performed by repeated-measures analysis of variance applying Bonferroni's correction factor (15) and within groups via repeated-measures analysis of variance and a multiple comparison test. Written informed consent was obtained from each subject. Results are expressed as means ± SE.

RESULTS

During the 10-h study, when subjects were allowed to sleep from 2430 to 0700, plasma glucose fell insignificantly from 5.1 mM at 2400 to 4.9 mM at 0800 (Fig. 1). As expected, the arterial-venous (A-V) glucose difference was minimal (0.1 mM) during the overnight fast. After the initial 2-h period required for isotopic equilibration, glucose utilization (R_d) fell with the onset of sleep from 2.15 ± 0.16 to 1.69 ± 0.15 mg · kg^{-1} · min^{-1} at 2430 and 0230, respectively ($P < .05$; Fig. 1). Glucose production (R_a) also fell from 2.19 ± 0.15 to 1.63 ± 0.14 mg · kg^{-1} · min^{-1} during the same period and remained significantly lower at each hour thereafter ($P < .05$). Despite the known sex differences in glucose homeostasis during a prolonged fast, glucose kinetics were similar in men and women (16). R_a fell during sleep in the men (n = 6) from 2.18 ± 0.16 to 1.65 ± 0.18 mg · kg^{-1} · min^{-1} and in the women (n = 5) from 2.08 ± 0.17 to 1.58 ± 0.27 mg · kg^{-1} · min^{-1}.

When examined in four of the subjects, the fall in R_a with the onset of sleep was positively correlated with a fall in heart rate (r = .76, $P < .002$) and core temperature (r = .80, $P < .001$), suggesting a relationship with lowered metabolic rate (Fig. 2). To determine whether the changes in glucose kinetics observed were due to sleep or were merely a reflection of the prolonged postabsorptive period, glucose kinetics were assessed in six additional subjects who were kept awake during the same period. Under these conditions, R_d and R_a fell modestly from 2.15 ± 0.08 to 1.85 ± 0.08 and from 2.15 ± 0.17 to 1.85 ± 0.08 mg · kg^{-1} · min^{-1} at 2430 and 0230, respectively (NS). Thereafter, both R_d and R_a increased to ~90% of baseline for the remainder of the fast. When expressed as percent of baseline, the mean fall in R_a observed during sleep was significantly attenuated in the control studies (24.0 vs. 9.0%, $P < .05$; Fig. 3). Similar differences were observed in R_d, thus preventing a rise in plasma glucose with the higher R_a (Fig. 4).

Possible explanations for the observed fall in glucose R_d and R_a include changes in insulin and counterregulatory hormones. After the evening meal (1800–1830), insulin levels fell to 5.3 ± 0.8 mU/L by 0100 and remained low throughout the night (Fig. 4). A similar fall in C-peptide concentrations

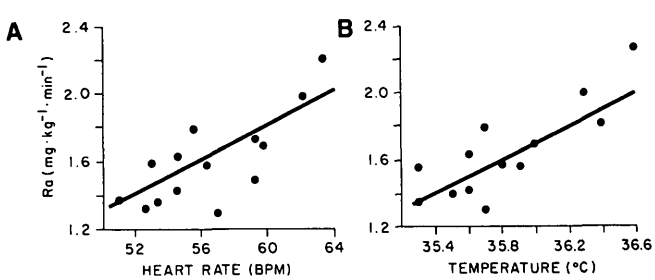

FIG. 2. Correlation between rates of glucose appearance (R_a) and heart rate (*A*; r = .76) and core temperature (*B*; r = .80) during overnight study in normal volunteers (n = 4). Subjects were allowed to sleep from 2430 to 0700.

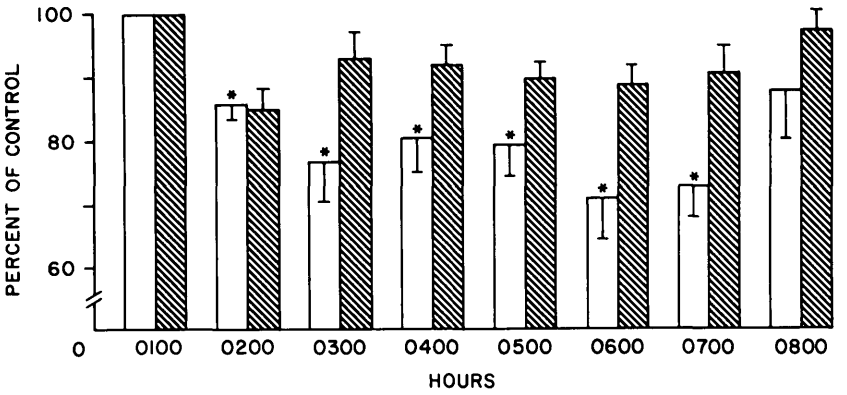

FIG. 3. Hourly rates of glucose appearance (R_a) during overnight study in presence (*open bars*) and absence (*hatched bars*) of sleep. Results are expressed as percent of baseline (mean of 2430 and 0100). Baseline R_a for sleeping subjects was 2.13 ± 0.11 mg · kg^{-1} · min^{-1} and for the awake subjects was 1.97 ± 0.10 mg · kg^{-1} · min^{-1}. Results are expressed as means ± SE. *$P < .05$ vs. baseline.

was noted during the study (0.39 ± 0.06 nM at 0100). We did not observe a rise in plasma glucose, insulin, or C-peptide during the final hours of the study in any of our subjects. Cortisol and growth hormone profiles during sleep were as previously reported (17; Fig. 5). A nocturnal rise in growth hormone was observed in the sleeping subjects when examined individually (mean peak growth hormone 0.43 ± 0.07 nM), but the nonsynchronous peaks were obscured when the values were averaged. Plasma glucagon concentrations were unchanged throughout the study (37.1 ± 6.5 pM at 2400 vs. 46.0 ± 9.0 pM at 0800). Similar profiles for plasma glucose, insulin, and C-peptide as well as for cortisol and glucagon were observed when sleep was prevented (Figs. 4 and 5). A nocturnal rise in growth hormone was not observed in the absence of sleep. The growth hormone profile in the subjects kept awake was consistently lower than that observed in the sleeping subjects, reflecting in part the greater growth hormone secretion of females participating in only the sleep studies (18; Fig. 5).

The potential role of selected gluconeogenic precursors (lactate, alanine, and glycerol) and FFA in the control of HGO during sleep was examined. A-V lactate differences examined in four subjects revealed a fall in the A-V lactate difference from −0.17 ± 0.04 mM at 2430 to −0.04 ± 0.02 mM at 0230 ($P < .05$). However, plasma venous lactate fell insignificantly in the total group (0.98 ± 0.13 mM at 0100 to a nadir of 0.83 ± 0.09 mM at 0600; Fig. 6). In the awake subjects, lactate levels were unchanged throughout the study. Plasma alanine levels were unchanged during the overnight study (0.36 ± 0.04 vs. 0.35 ± 0.04 mM at 0100 and 0800, respectively). Plasma glycerol concentrations fell from 0.06 ± 0.01 during the baseline period to 0.04 ± 0.01 mM at 0400 ($P < .05$) and remained significantly depressed thereafter in the sleeping subjects (Fig. 6). No fall in glycerol was observed in the subjects kept awake. Plasma FFA was determined in seven of the sleeping subjects (insufficient sample was available from the first 4 subjects studied) and in all of the subjects kept awake. A significant fall in FFA was observed in the sleeping subjects from 0100 to 0300 (0.56 ± 0.02 vs. 0.39 ± 0.03 mM, $P < .05$), which was positively correlated with R_a ($r = .87$, $P < .05$). Plasma FFA remained depressed during the sleep study. However, as observed for glycerol, no significant fall in FFA was observed when sleep was prevented (Fig. 6).

DISCUSSION

A fall in HGO was observed during sleep in subjects fasted overnight. HGO fell synchronously with a reduction in peripheral glucose R_d, resulting in a minimal change in plasma glucose. The fall in R_d, which we believe to be primary, may be partly explained by decreased muscle glycolysis as a result of reduced activity during sleep. A fall in adrenergic

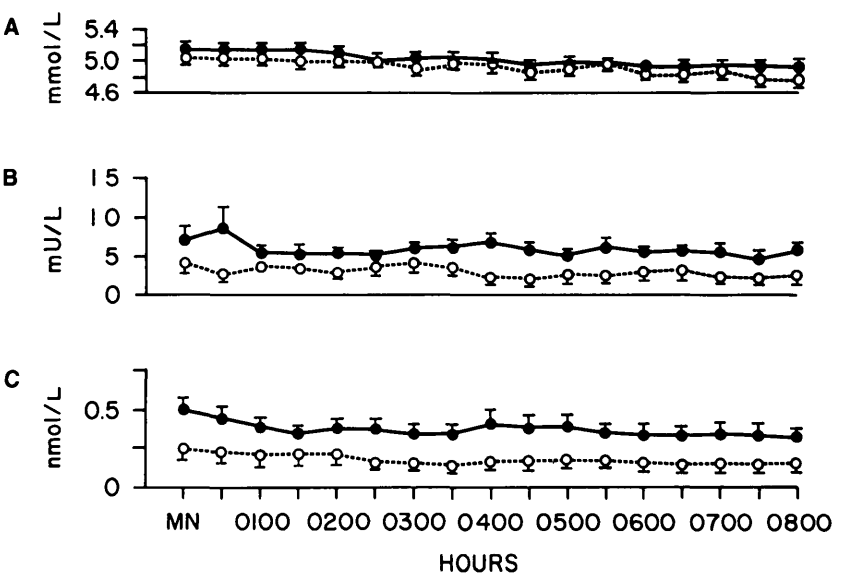

FIG. 4. Plasma glucose (*A*), insulin (*B*), and C-peptide (*C*) levels during overnight study in normal volunteers. Eleven subjects were allowed to sleep from 2430 to 0700 (●); 6 subjects were kept awake during same period (○). Results are expressed as means ± SE.

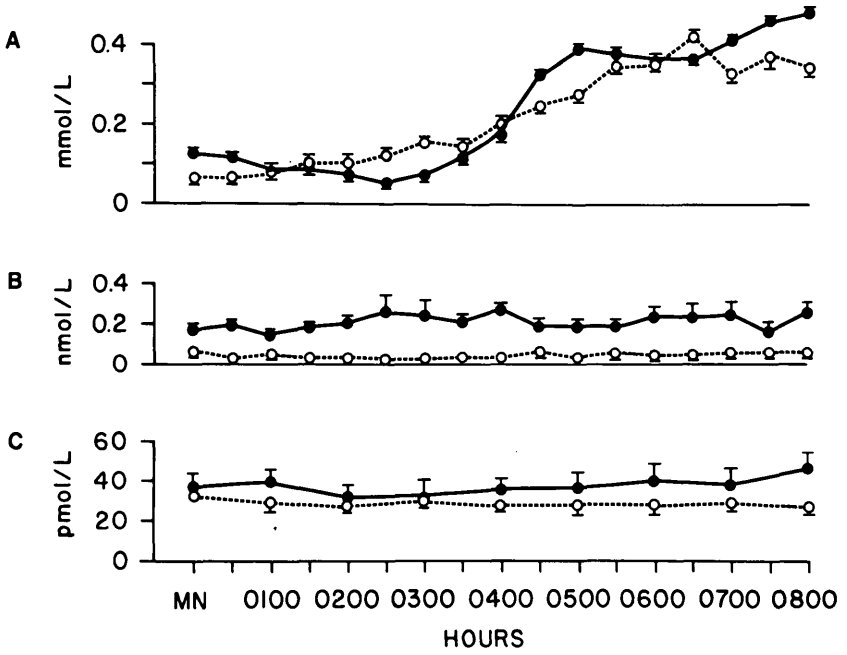

FIG. 5. Plasma cortisol (*A*), growth hormone (*B*), and glucagon (*C*) levels during overnight study in normal volunteers. Eleven subjects were allowed to sleep from 2430 to 0700 (●); 6 subjects were kept awake during same period (○). Results are expressed as means ± SE.

firing rate, perhaps centrally mediated, could also contribute to decreased R_d by reducing glycolysis in tissues responsible for thermogenesis, e.g., adipose tissue and skin. Catecholamines are known to increase glycolysis in adipose tissue (19). Although adrenergic firing rate has not been specifically examined during sleep in humans, the fall in firing rate that occurs during anesthesia suggests by analogy what is intuitively likely (20). Furthermore, the fall in plasma glycerol and FFA observed in this study may reflect diminished lipolysis as a result of a decrease in sympathetic activity. Although the fall in R_d during sleep may be mediated by a decrease in neural and motor activity, a hormonal or metabolic signal to reduce HGO in response to decreased peripheral R_d is more consonant with our concepts of fine metabolic control (21).

The reduction in HGO during sleep paradoxically occurred while insulin secretion fell, as reflected by reduced C-peptide and insulin levels. The possibility that changes in counterregulatory hormones might be responsible for the fall in HGO was considered. The decline in serum cortisol levels from 2400 to 0230 potentially could have reduced HGO, because glucocorticoids are known insulin antagonists. However, the physiologic rise in serum cortisol levels between 0400 and 0800 did not result in a similar rise in HGO, as might have been expected if glucocorticoids had been responsible for the sleep-associated change in HGO. Furthermore, serum cortisol levels in the control (awake) group were not different from the study group despite a marked difference in HGO. In addition to its prolonged insulin-antagonist effect, growth hormone also has an early, transient insulinlike effect that could conceivably produce a fall in HGO (22). However, no increase in glucose clearance was observed to support such a mechanism. Glucagon levels fluctuated minimally throughout the study and therefore probably played no role in the sleep-associated reduction in HGO. Plasma catecholamine levels (not measured in this study) change minimally during early sleep (23), and changes of this magnitude are unlikely to influence R_a significantly (24). More important, catecholamine levels remain low when nocturnal sleep is prevented (25), a condition under which HGO did not fall in this study.

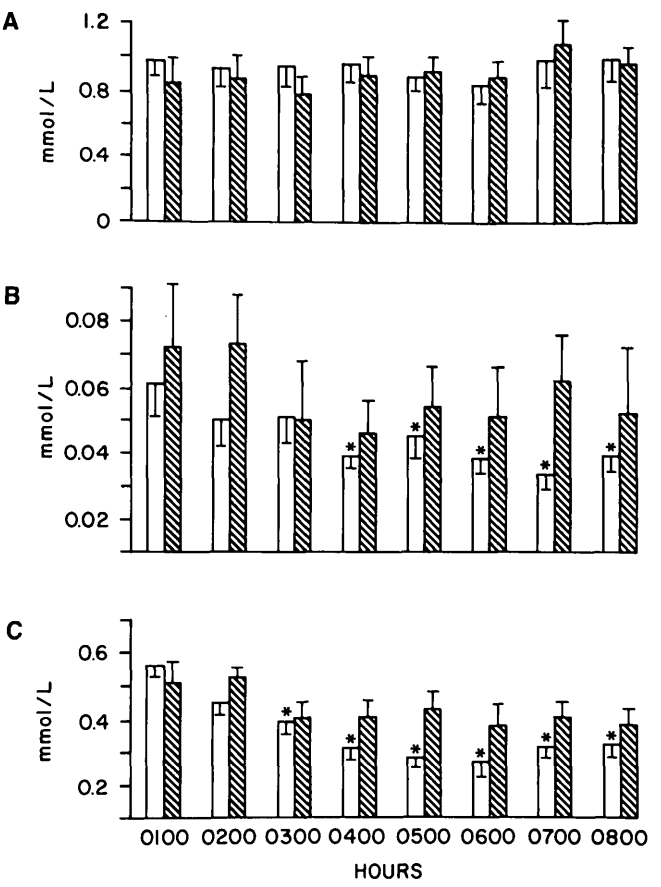

FIG. 6. Mean hourly lactate (*A*), glycerol (*B*), and free–fatty acid (*C*) concentrations during overnight study in presence (*open bars*; $n = 11$) and absence (*hatched bars*; $n = 6$) of sleep. Results are expressed as means ± SE. *$P < .05$ vs. baseline.

Gluconeogenic substrates (lactate, alanine, and glycerol) and FFA were also examined during sleep. Despite a significant fall in the A-V lactate difference (4 subjects), probably due to reduced forearm lactate production (26), venous lactate and alanine levels were not significantly reduced overnight in either the sleeping or awake subjects. However, a significant fall in both plasma glycerol and FFA occurred overnight during sleep. Despite the reduction in plasma glycerol, its 3% contribution to gluconeogenesis in the postabsorptive state makes limited availability of this substrate an unlikely explanation for the reduction in HGO (27). Substrate turnover was not determined. However, a reduction in gluconeogenic substrate production or hepatic extraction sufficient to explain a 25% reduction in HGO seems unlikely, particularly because 75% of HGO after a 14-h fast results from glycogenolysis (28,29).

Because reduced substrate availability is an unlikely explanation for the fall in R_a overnight during sleep, we can only speculate on the nature of the putative signal reducing HGO in response to decreased peripheral R_d and lipolysis. The virtually synchronous changes in peripheral glycerol and FFA concentration and HGO suggest a relationship between the two. Both glycerol and FFA (as well as lactate) have been shown to inversely modulate fructose 2,6-bisphosphate (F-2,6-P_2) concentrations (30–32). The fall in delivery of these signals to the liver might increase F-2,6-P_2, thus favoring glycolysis over gluconeogenesis and perhaps shunting glucose 6-phosphate (G-6-P) formed from liver glycogen into the glycolytic pathway versus release as glucose. That glycerol and FFA are the more likely candidates for the peripheral signal than lactate is suggested by the correlation between R_a, FFA, and glycerol in the two study groups. Although hormones (glucagon and insulin) may control the breakdown of liver glycogen to G-6-P, intermediary metabolites reflecting peripheral metabolism may synergistically modulate the effect of these hormones on F-2,6-P_2. In this manner, peripheral metabolism may determine the fate of G-6-P so generated.

Our findings are similar to those of Ravussin et al. (30), who have demonstrated, via indirect calorimetry, a 19% decrease in energy expenditure during sleep. These authors suggest that both the duration and quality of sleep are determinants of energy expenditure. Using isotopic glucose dilution, we noted a 25% fall in glucose R_d and R_a during sleep. Thus, reported "basal" levels of HGO (1.8–2.2 mg · kg^{-1} · min^{-1}) do not represent minimal daily HGO values because HGO fell from basal levels to 1.60 mg · kg^{-1} · min^{-1} during sleep in this study. Even the modest increase in activity associated with sleeplessness was sufficient to attenuate the fall in HGO and lipolysis. In addition, the fall in HGO during sleep and its subsequent rise to basal production rates on arousal have important implications for the dawn phenomenon. This study failed to support a dawn phenomenon in normal humans in that plasma glucose and immunoreactive insulin were unchanged at 0600 compared with midnight levels. Plasma glucose did not change in our studies because R_a and R_d changed synchronously. As discussed in "Perspectives in Diabetes" (this issue, p. 273), the nocturnal changes in glucose in diabetic patients are probably due to a mismatch between R_a and R_d in those subjects.

These studies support the concept that energy expenditure and glucose metabolism are closely linked in normal humans and that the fall in metabolic requirements and glucose R_d associated with sleep are matched by a fall in R_a. Synchronous changes in R_d and R_a and the close correlation between peripheral FFA and glycerol production and HGO suggest a peripherally generated signal affecting HGO. However, we cannot exclude the possibility that the fall in R_d and changes in peripherally derived metabolites as well as the fall in HGO are mediated solely by central mechanisms.

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REFERENCES

1. Saudek CD, Felig P: The metabolic events of starvation. *Am J Med* 60:117–26, 1976
2. Achou CJ, Hetenyi G: Is the peripheral utilization of glucose a regulator of hepatic glucose production and of glycemia? *Can J Physiol Pharmacol* 52:158–65, 1974
3. DeFronzo RA, Ferrannini E, Hendler R, Felig P, Wahren J: Regulation of splanchnic and peripheral glucose uptake by insulin and hyperglycemia in man. *Diabetes* 32:35–45, 1983
4. Wahren J, Felig P, Ahlborg G, Jorfeldt L: Glucose metabolism during leg exercise in man. *J Clin Invest* 50:2715–25, 1971
5. Brobeck JR: Energy exchange. In *Medical Physiology*. Mountcastle VB, Ed. St. Louis, MO, Mosby, 1974, p. 1247–51
6. Morgan CR, Lazarow A: Immunoassay of insulin: two antibody system. *Diabetes* 12:115–26, 1963
7. Kumar MS, Schumacher OP, Deodhar SD: Measurement of serum C-peptide immunoreactivity by radioimmunoassay in insulin-dependent diabetics. *Am J Clin Path* 74:78–82, 1980
8. Schalch DS, Parker ML: A sensitive double antibody immunoassay for human growth hormone in plasma. *Nature* (Lond) 203:1141–42, 1963
9. Aquilar-Parada E, Eisentraut AM, Unger RH: Pancreatic glucagon secretion in normal and diabetic subjects. *Am J Med Sci* 257:415–19, 1969
10. Miles J, Glasscock R, Aikens J, Gerich J, Haymond M: A microfluorometric method for the determination of free fatty acids in plasma. *J Lipid Res* 24:96–99, 1983
11. Noll F: Determination of L-lactate with LDH, GPT and NAD. In *Methods of Enzymatic Analysis*. Bergmeyer HU, Ed. New York, Academic, 1974, p. 1475–79
12. Wieland O: Enzymatic analysis of glycerol. In *Methods of Enzymatic Analysis*. Bergmeyer HU, Ed. New York, Academic, 1974, p. 1404–409
13. DeBodo RC, Steele R, Altszuler N, Dunn A, Bishop JS: On the hormonal regulation of carbohydrate metabolism: studies with ^{14}C-glucose. *Recent Prog Horm Res* 19:445–88, 1963
14. Cowan JS, Hetenyi G: Glucoregulatory responses in normal and diabetic dogs recorded by a new tracer method. *Metabolism* 20:360–72, 1971
15. Feller W: *An Introduction to Probability Theory and Its Applications*. 2nd ed. New York, Wiley, 1957
16. Merimee TJ, Tyson JE: Stabilization of plasma glucose during fasting. *N Engl J Med* 291:1275–78, 1974
17. Mauras N, Rogol AD, Clarke WL: Failure to detect the "dawn phenomenon" in nondiabetic subjects with markedly different patterns of nocturnal growth hormone secretion. *J Clin Endocrinol Metab* 62:975–79, 1986
18. Merimee TJ, Burgess JA, Rabinowitz D: Sex-determined variation in serum insulin and growth hormone response to amino acid stimulation. *J Clin Endocrinol Metab* 26:791–93, 1966
19. Rodbell M: Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. *J Biol Chem* 239:375–80, 1964
20. Delius W, Hagbarth K-E, Hongell A, Wallin BG: Maneuvers affecting sym-

pathetic outflow in human skin nerves. *Acta Physiol Scand* 84:177–86, 1972
21. Hue L: Futile cycles and regulation of metabolism. In *Metabolic Compartments*. Sies H, Ed. London, Academic, 1982, p. 71–97
22. MacGorman LR, Rizza RA, Gerich JE: Physiological concentrations of growth hormone exert insulin-like and insulin antagonistic effects on both hepatic and extrahepatic tissues in man. *J Clin Endocrinol Metab* 53:556–59, 1981
23. Bolli GB, De Feo P, De Cosmo S, Perriello G, Ventura MM, Calcinaro F, Lolli C, Campbell P, Brunetti P, Gerich JE: Demonstration of a dawn phenomenon in normal human volunteers. *Diabetes* 33:1150–53, 1984
24. Campbell PJ, Bolli GB, Cryer PE, Gerich JE: Pathogenesis of the dawn phenomenon in patients with insulin-dependent diabetes mellitus. *N Engl J Med* 312:1473–79, 1984
25. Akerstedt T, Gillberg M: Circadian variation of catecholamine excretion and sleep. *Eur J Appl Physiol* 51:203–10, 1983
26. Jackson RA, Roshania RD, Hawa MI, Sim BM, DiSilvio L: Impact of glucose ingestion on hepatic and peripheral glucose metabolism in man: an analysis based on simultaneous use of the forearm and double isotope techniques. *J Clin Endocrinol Metab* 63:541–49, 1986
27. Nurjhan N, Campbell PJ, Kennedy FP, Miles JM, Gerich JE: Insulin dose-response characteristics for suppression of glycerol release and conversion to glucose in humans. *Diabetes* 35:1326–31, 1986
28. Dietze G, Wicklmayr M, Hepp KD, Bogner W, Mehnert H, Czempiel H, Henftline HG: On gluconeogenesis of human liver. *Diabetologia* 12:555–61, 1976
29. Consoli A, Kennedy F, Miles J, Gerich JE: Determination of Krebs cycle metabolic carbon exchange in vivo and its use to estimate the individual contributions of gluconeogenesis and glycogenolysis to overall glucose output in man. *J Clin Invest* 80:1303–10, 1987
30. Chaekal O, Borz JC, Sugano T, Harris RA: Role of fructose-2,6-bisphosphate in the regulation of glycolysis and gluconeogenesis in chicken liver. *Arch Biochem Biophys* 225:771–78, 1983
31. Uyeda K, Furuya E, Richards CS, Yokohama M: Fructose-2,6-P_2, chemistry and biological function. *Mol Cell Biochem* 48:97–120, 1984
32. Hue L, Rider MH: Role of fructose 2,6-bisphosphate in the control of glycolysis in mammalian tissues. *Biochem J* 245:313–24, 1984
33. Ravussin E, Lillioja S, Anderson TE, Clingtin L, Bogardus C: Determinant of 24-hour energy expenditure in man: method and result using a respiratory chamber. *J Clin Invest* 78:1568–78, 1986