Intranasal insulin decreases circulating cortisol concentrations during early sleep in elderly humans

Subtitle: Insulin and sleep-related HPA axis activity

Matthias Thienel, MSc[1]; Ines Wilhelm, PhD[2]; Christian Benedict, PhD[3]; Jan Born, PhD[1,4,5]; Manfred Hallschmid, PhD[1,4,5]

[1]Department of Medical Psychology and Behavioral Neurobiology, University of Tübingen, Germany; [2]Child Development Center, University Children's Hospital Zurich, Zurich, Switzerland; [3]Department of Neuroscience, Uppsala University, Uppsala, Sweden; [4]German Center for Diabetes Research (DZD), Tübingen, Germany; [5]Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at the University of Tübingen (IDM), Tübingen, Germany

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Corresponding author

Manfred Hallschmid, PhD
Department of Medical Psychology and Behavioral Neurobiology
University of Tübingen
Otfried-Müller-Str. 25
72076 Tübingen, Germany
Phone: +49-7071-2988925
Fax: +49-7071-2925016
Email: manfred.hallschmid@uni-tuebingen.de

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Abstract

Study Objectives. Aging is associated with maladaptive increases in hypothalamic-pituitary-adrenal (HPA) axis activity that can predispose to or aggravate metabolic and cognitive disorders. Nadir concentrations of adrenocorticotropin (ACTH) and cortisol in the first night-half are particularly sensitive indicators of baseline HPA axis activity. We investigated the effect of central nervous insulin administration via the intranasal pathway on ACTH and cortisol concentrations during early sleep in elderly and young subjects.

Methods. In double-blind within-subject comparisons, intranasal insulin (160 IU) or placebo was administered to 14 elderly (mean age 70.0 years; 6/8 women/men) and 30 young (23.6 years; 14/16) healthy, unmedicated subjects at 2230 h, i.e., 30 min before bedtime. Sleep was polysomnographically assessed and blood samples were drawn for the determination of ACTH, cortisol, insulin, C-peptide, and blood glucose.

Results: Elderly compared to young participants displayed increased first night-half cortisol concentrations ($P < 0.04$) and reductions in slow wave and REM sleep ($P < 0.001$). In the elderly subjects, intranasal insulin compared to placebo reduced serum cortisol levels by around 15.30 nmol/l between 2300 h and 0020 h ($P = 0.03$), but remained without effect in young participants ($P > 0.56$). There were no systematic effects on other parameters.

Conclusions: Intranasal insulin attenuates HPA axis activity during early sleep in healthy elderly but not young subjects. This finding supports the concept of central insulin as an inhibitory signal in the control of HPA axis activity and suggests that improving brain insulin signaling could attenuate sleep-associated stress axis activity in older age.

Keywords: Insulin, brain, sleep, aging, hypothalamic-pituitary-adrenal axis, cortisol.

Statement of Significance

Changes in sleep-associated neuroendocrine secretion patterns that emerge in older age are assumed to be pathophysiological factors in metabolic and cognitive impairments. Increased activity of the hypothalamo-pituitary-adrenal (HPA) stress axis, which follows a circadian pattern with nadir values during early sleep, may be particularly detrimental. We show that insulin administered to the brain via the intranasal pathway before sleep reduces circulating cortisol concentrations in elderly but not in young subjects during the first night-half. While we did not examine long-term effects of pre-sleep insulin administration, findings indicate an inhibitory role of brain insulin signaling in sleep-associated HPA axis regulation, and suggest that central insulin delivery might be useful in normalizing increased stress axis activity in elderly humans.

Introduction

The efficient regulation of neuroendocrine stress systems including the hypothalamic-pituitary-adrenal (HPA) axis is relevant not only with regard to their activation in response to environmental challenges, but also to endogenous circadian rhythms. Release of adrenocorticotropin (ACTH) and cortisol is triggered by corticotropin-releasing hormone (CRH) and regulated via glucocorticoid feedback at the hippocampal and hypothalamic levels. In the first night-half, ACTH and cortisol concentrations reach nadir values which indicate basal secretory HPA axis activity in the absence of external stimulation. Elderly humans typically display changes in sleep-related neuroendocrine patterns, in particular a decrease in growth hormone release and an increase in HPA axis activity, which are paralleled by a reduction in the amount of time spent in rapid eye-movement (REM) and slow-wave sleep.[1,2] Since nocturnal hypercortisolism in aging individuals is assumed to contribute to disorders such as obesity, depression, and cognitive impairments,[3] identifying non-glucocorticoid factors that inhibit HPA axis activity may contribute to new approaches in the prevention and treatment of these ailments. Insulin has been repeatedly shown to attenuate HPA axis secretion during wakefulness when administered to the brain via the intranasal pathway.[4–6] We therefore investigated the effect of intranasal insulin on HPA axis secretion during early sleep in healthy elderly compared to young subjects. Considering the association between increased age and nocturnal HPA axis hyperactivity[1] as well as impaired central nervous insulin signaling,[7] we expected an inhibitory effect of insulin on ACTH and cortisol secretion particularly in elderly subjects.

Materials and Methods

Participants. Fourteen healthy elderly volunteers (8 men, 6 women; age, mean ± SEM, 70.00 ± 0.63 years; BMI, 24.83 ± 0.66 kgm^{-2}) and 30 healthy young individuals (16 men, 14 women; age, 23.63 ± 0.45 years; BMI, 22.93 ± 0.33 kgm^{-2}) participated in, respectively,

Experiments I and II. Data of the young participants were collected in experiments on sleep-related memory function (see ref. 8 for respective results and detailed information). Participants reported having a regular sleep-wake cycle and were not on medication except for estrogen-dominant oral contraceptives taken by all young women. All subjects spent one adaptation night in the sleep laboratory. They gave written informed consent to the study that conformed to the Declaration of Helsinki and was approved by the local ethics committee.

Study design and procedure. Both experiments followed a balanced, placebo-controlled, double-blind, within-subject, crossover design. All participants took part in two sessions which were identical except for the administration of insulin or placebo. Sessions were scheduled to be apart as close to 28 days as possible and the young women did not participate during their menstruation phases. Experiments started at 1800 h with a light meal. Electrodes were attached for standard polysomnographic recordings, including electroencephalogram (at sites C3 and C4), electrooculogram and electromyogram. Polysomnographic recordings were scored offline according to standard criteria as wake, sleep stages N1, N2, N3, and REM sleep. At 2230 h, before going to sleep, subjects were intranasally administered a total dose of 1.6 ml insulin (160 IU; Insulin Actrapid; Novo Nordisk, Mainz, Germany) or vehicle via 16 0.1 ml puffs (8 per each nostril) in 1-min intervals. Subjects were allowed to sleep between 2300 h (lights off) and 0700 h (awakening).

Blood sampling. Peripheral blood for the assessment of serum cortisol, C-peptide, insulin, as well as glucose and plasma ACTH was sampled during a pre-sleep baseline and at 20-min intervals during the first night-half until 0320 h (cf. Figure 1). Blood was drawn via long thin tubes enabling blood collection from an adjacent room while minimizing disruptive effects on the subject's sleep. Routine assays were used to determine concentrations of ACTH, cortisol, C-peptide (all Immulite, DPC, Los Angeles, CA), insulin (Insulin ELISA Kit, Dako, Glostrup, Denmark), and glucose (HemoCue Glucose 201 Analyzer, HemoCue AB, Ångelholm, Sweden).

Control measures. Appetite, thirst, and sleepiness were repeatedly self-reported on visual analogue scales (0-100 mm). Mood and well-being were assessed via self-rating scales covering positive/negative affect as well as alertness throughout the experiments.

Statistical Analyses. For the analysis of sleep stages, one female and one male participant of Experiment II were excluded because of data loss. Analyses relied on analyses of covariance (ANCOVA) for repeated measurements with baseline values as covariates and the between subject-factor 'sex' (male/female) and the within-subject factors 'treatment' (insulin/placebo) and 'time'. Degrees of freedom were Greenhouse-Geisser-corrected. Areas under the curve (AUC) according to the trapezoidal rule and single time points were compared by *t*-tests. For comparisons between elderly and young subjects, linear mixed models were used with the between-subject factor age (young/elderly). In addition, individual slope coefficients were obtained in the form of beta weights of linear regression lines fitted to ACTH and cortisol values between 2300-0320 h, and were compared between groups by two-tailed unpaired *t*-tests. A *P*-value < 0.05 was considered significant; data are presented as means ± SEM.

Results

Cortisol $AUC_{2300\text{-}0320}$ values were higher in elderly compared to young subjects (13,472 ± 584 vs. 11,034 ± 972 nmol/l*min, $t(41) = -2.22$, $P = 0.032$; $t(42) = -0.74$, $P = 0.463$ for respective ACTH values). Accordingly, the increases in ACTH and cortisol concentrations emerging across the first night-halves of the respective placebo conditions were stronger in elderly compared to young subjects (beta weight means, ACTH, 0.15 ± 0.02 vs. 0.06 ± 0.01, $t(19) = -3.48$, $P = 0.003$; cortisol, 6.81 ± 1.66 vs. 0.64±1.21, $t(41) = -2.96$, $P = 0.005$). Nadir values of ACTH and cortisol did not differ between groups regarding levels (all $P > 0.20$) and timing ($P > 0.24$).

Blood parameters did not differ between conditions during baseline (all $P \geq 0.15$). In the elderly subjects, insulin compared to placebo administration decreased cortisol

concentrations during the first night half (2300-0320 h; ($F(1,10) = 5.83$, $P = 0.036$ for treatment; $t(13) = 2.40$, $P = 0.03$ for the difference in $AUC_{2300\text{-}0020\ h}$), whereas this effect was absent in young participants (all $P > 0.44$; $F(22, 129) = 2.23$, $P = 0.003$ for treatment × time × age; Figure 1A). In the elderly, the insulin-induced decrease in cortisol concentrations emerged irrespective of the subjects' sex ($P > 0.32$). Its extent was proportional to the respective cortisol nadir level in the placebo condition (r = 0.60, $P = 0.03$, Pearson's coefficient), but was statistically unrelated to changes in nocturnal levels of insulin, C-peptide, and glucose (all $P > 0.38$; $P > 0.46$ for the group of young subjects). Plasma ACTH levels were comparable between groups ($P = 0.13$) and were not influenced by treatment (both $P \geq 0.56$ for treatment; all $P \geq 0.10$ for single time point comparisons; Figure 1B).

Serum insulin concentrations were not affected by insulin administration in the elderly subjects (all $P \geq 0.58$). In the young participants they rose shortly after substance administration but were comparable between conditions thereafter ($P \geq 0.73$ for treatment × time; Figure 1C, upper lines), with no statistical differences to the group of elderly subjects ($P = 0.24$ for age). In both groups, serum C-peptide concentrations slightly decreased after intranasal insulin administration (both $P < 0.1$ for differences at 2320 h), but did not differ between conditions thereafter ($P = 0.68$ and $P = 0.85$, respectively, for treatment × time; $P > 0.62$ for age). In accordance with the ephemeral increase in peripheral insulin concentrations, in the group of young subjects blood glucose levels were acutely decreased after peptide administration at 2300 h, but subsequently returned to placebo condition values ($P = 0.65$ for treatment × time). Across conditions, blood glucose levels were lower in elderly than young individuals ($P < 0.001$ for age; Figure 1C, lower lines).

Intranasal insulin compared to placebo generally did not alter whole-night sleep architecture and total sleep time (all $P > 0.29$). Early sleep (2300 h-0320 h) likewise was unaffected by insulin in the elderly ($P > 0.51$) and young subjects ($P > 0.29$). Independent of

treatment, elderly in comparison to younger subjects had longer wake and light sleep (N1) periods at the expense of slow wave (N3) and REM sleep ($F(2,92) = 29.78$, $P < 0.001$ for sleep stage × age group; Table S1). Self-rated mood as well as hunger, thirst, and sleepiness ratings were not affected by insulin (all $P \geq 0.15$).

Discussion

We show that intranasal insulin reduces circulating concentrations of cortisol during the first night-half in elderly subjects who in comparison to young controls display increased secretory HPA axis activity. In line with previous observations,[1] elderly subjects also displayed an increased amount of time spent awake and in sleep stage 1, but a decreased duration of REM sleep and sleep stage 3. Intranasal insulin delivery to the brain did not induce significant changes in sleep architecture, which accords with the lack of respective effects of peripheral hyperinsulinemia.[9] The inhibitory influence of brain insulin delivery on HPA axis secretory activity extends reports by our and other groups that (subchronic) intranasal insulin treatment attenuates HPA axis activity.[4–6] Physiologically, circulating insulin reaches the CNS via a receptor-mediated saturable transport across the blood-brain barrier and might dampen HPA axis secretion by acting on hypothalamic nuclei and limbic structures like the hippocampal formation, which express large numbers of insulin receptors.[10] In contrast, systemic hyperinsulinemia during euglycemic hyperinsulinemic clamps rather increases HPA axis secretion,[11] probably by boosting hormone synthesis in the adrenal cortex that is not effectively reached by intranasal insulin.

Notably, central nervous insulin delivery reduced early-sleep cortisol concentrations in elderly but not in young participants. It seems unlikely that the small amount of intranasal insulin reaching the blood stream via spillover and causing a short drop in plasma glucose levels masked a centrally inhibiting insulin effect in the younger subjects by stimulating cortisol release. Spillover-induced increases in circulating insulin concentrations are

negligible[12] compared to the elevations needed to stimulate HPA axis activity under euglycemic conditions.[11] Moreover, blood glucose levels remained clearly above the hypoglycemic threshold of 3.6-3.8 mmol/l where hormonal counterregulation sets in. Although treatment effects on ACTH concentrations were not detected, the fact that changes in cortisol were generally unrelated to changes in parameters of peripheral glucose homeostasis points to a central nervous mediation of insulin's suppressive effect on sleep-related HPA axis secretion in elderly subjects.

The preponderance of the intranasal insulin effect in our elderly subjects accords with previous studies indicating that in young, healthy men, attenuating effects of intranasal insulin on basal HPA axis activity only emerge after long-term administration,[5] whereas the peptide acutely dampens cortisol concentrations in young men confronted with a psychological stressor.[6] Acutely attenuating effects on basal cortisol concentrations during wakefulness were found in obese,[4] but not in normal-weight men.[5] Obesity is associated with and also appears to be promoted by excessive HPA axis secretion, e.g., due to chronic stress.[13] Normal aging likewise goes along with alterations in the regulation of the HPA system, in particular increased nocturnal cortisol secretion.[1] Our sample of elderly men and women displayed a high level of physical health and only moderate signs of nocturnal HPA axis up-regulation, which might explain the relative subtlety of the observed insulin effect. Still, the extent of insulin-induced cortisol reductions was positively related to the height of respective cortisol nadir levels. Therefore, inhibitory effects of insulin on HPA axis secretory activity are expected to be stronger in aging subjects who show a higher degree of stress axis disinhibition. In these subjects, intranasal insulin may be a helpful means to normalize nocturnal HPA axis activity, an effect which might furthermore contribute to the beneficial metabolic and cognitive impact of nervous insulin delivery.[14]

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References

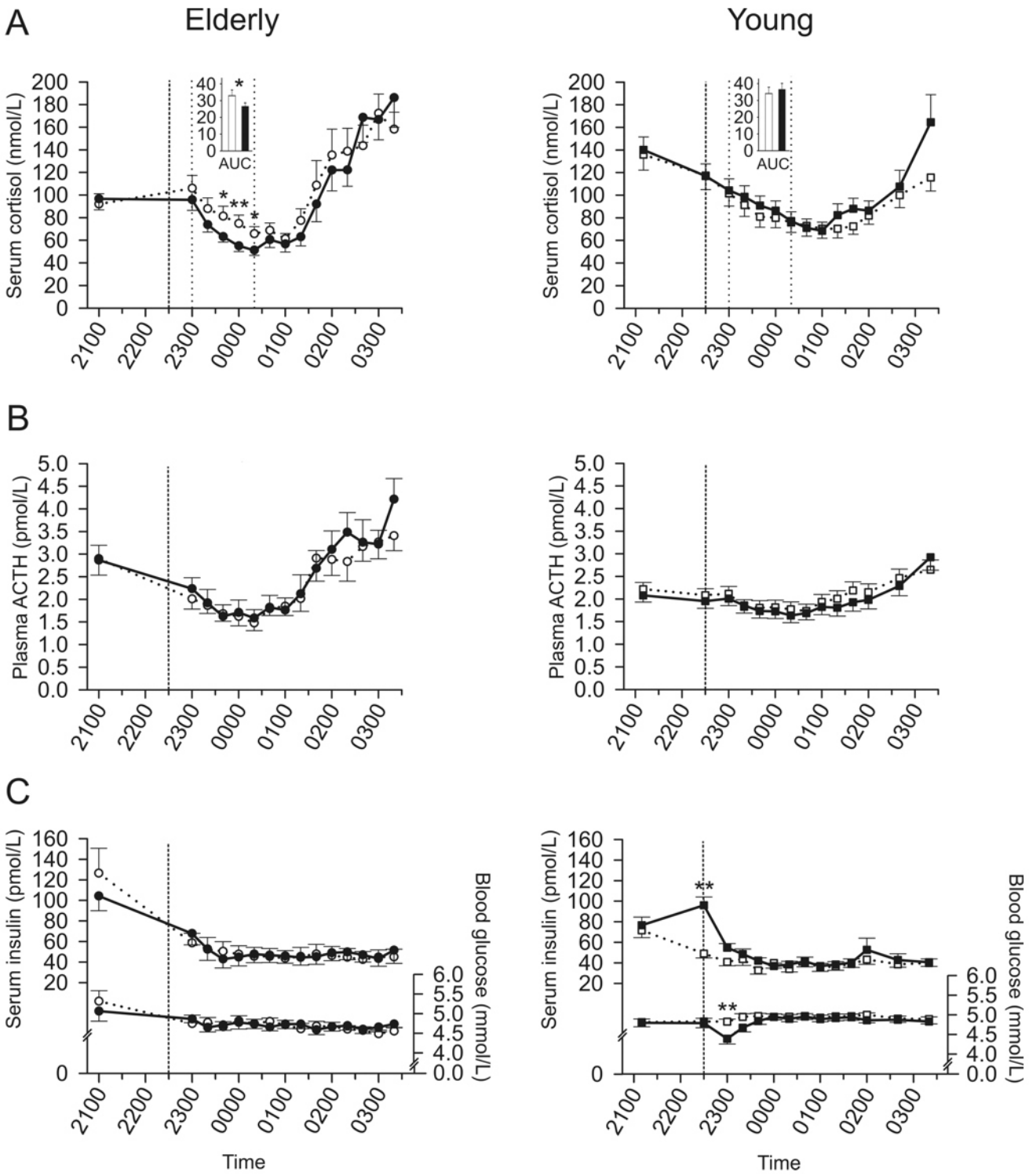
1. Van Cauter E, Leproult R, Plat L. Age-related changes in slow wave sleep and REM sleep and relationship with growth hormone and cortisol levels in healthy men. JAMA 2000;284:861-8.
2. Ohayon MM, Carskadon MA, Guilleminault C, Vitiello M V. Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: developing normative sleep values across the human lifespan. Sleep 2004;27:1255-73.
3. McEwen BS. The neurobiology of stress: from serendipity to clinical relevance. Brain Res 2000;886:172-89.
4. Hallschmid M, Benedict C, Schultes B, Born J, Kern W. Obese men respond to cognitive but not to catabolic brain insulin signaling. Int J Obes (Lond) 2008;32:275-82.
5. Benedict C, Hallschmid M, Hatke A, et al. Intranasal insulin improves memory in humans. Psychoneuroendocrinology 2004;29:1326-34.
6. Böhringer A, Schwabe L, Richter S, Schachinger H. Intranasal insulin attenuates the hypothalamic-pituitary-adrenal axis response to psychosocial stress. Psychoneuroendocrinology 2008;33:1394-400.
7. Biessels GJ, Reagan LP. Hippocampal insulin resistance and cognitive dysfunction. Nat Rev Neurosci 2015;16:660-71.
8. Feld GB, Wilhelm I, Benedict C, et al. Central nervous insulin signaling in sleep-associated memory formation and neuroendocrine regulation. Neuropsychopharmacology 2016;41:1540-50.
9. Sturis J, Scheen AJ, Leproult R, Polonsky KS, van Cauter E. 24-hour glucose profiles during continuous or oscillatory insulin infusion. Demonstration of the functional significance of ultradian insulin oscillations. J Clin Invest 1995;95:1464-71.
10. Plum L, Schubert M, Brüning JC. The role of insulin receptor signaling in the brain. Trends Endocrinol Metab 2005;16:59-65.
11. Fruehwald-Schultes B, Kern W, Bong W, et al. Supraphysiological hyperinsulinemia acutely increases hypothalamic-pituitary-adrenal secretory activity in humans. J Clin Endocrinol Metab 1999;84:3041-6.
12. Ott V, Lehnert H, Staub J, Wönne K, Born J, Hallschmid M. Central nervous insulin administration does not potentiate the acute glucoregulatory impact of concurrent mild hyperinsulinemia. Diabetes 2015;64:760-5.
13. Incollingo Rodriguez AC, Epel ES, White ML, Standen EC, Seckl JR, Tomiyama AJ. Hypothalamic-pituitary-adrenal axis dysregulation and cortisol activity in obesity: A systematic review. Psychoneuroendocrinology 2015;62:301-18.
14. Spetter MS, Hallschmid M. Intranasal neuropeptide administration to target the human brain in health and disease. Mol Pharm 2015;12:2767-80.

Table S1. Sleep stages.

	Elderly (mean ± SEM)			Young (mean ± SEM)			
	Insulin	**Placebo**	***P***	**Insulin**	**Placebo**	***P***	***P* (group)**
Total sleep (min)	456.57 ± 11.12	458.39 ± 5.68	0.89	461.59 ± 3.37	460.70 ± 4.57	0.84	0.57
Wake (%)	17.68 ± 2.36	15.36 ± 2.21	0.46	1.29 ± 0.31	1.33 ± 0.34	0.92	<0.001
N1 (%)	12.36 ± 1.37	11.34 ± 1.11	0.28	6.94 ± 0.73	6.86 ± 0.93	0.91	<0.001
N2 (%)	51.64 ± 2.97	53.61 ± 2.16	0.34	54.44 ± 1.36	55.01 ± 1.60	0.64	0.41
N3 (%)	5.40 ± 1.27	6.15 ± 1.42	0.27	15.89 ± 0.98	15.72 ± 0.97	0.83	<0.001
REM (%)	12.87 ± 1.48	13.49 ± 0.88	0.74	20.47 ± 0.83	19.98 ± 1.00	0.65	<0.001

Mean (±SEM) total sleep time and time spent in different sleep stages (relative to total sleep period). *P*-values derive from comparisons between conditions (*t*-tests) and group ANOVA main effects; elderly subjects, $n = 14$; young subjects, $n = 28$.

Figure 1. Blood parameters. Mean ± SEM concentrations of serum cortisol (**A**; inserts depict $AUC_{2300\text{-}0020\ h}$ values in (nmol/l*min)/100), plasma ACTH (**B**), serum insulin and blood glucose (**C**; upper and lower lines, respectively) measured in elderly (n = 14; left panels) and young participants (n = 30; right panels) who were intranasally administered insulin (160 IU; black dots/squares/bars and solid lines) or placebo (white dots/squares/bars and dotted lines) at 2230 h (dotted line). *P < 0.05, **P < 0.01 for comparisons between groups (t tests).


A
Elderly
Young
Serum cortisol (nmol/L)
AUC
B
Plasma ACTH (pmol/L)
C
Serum insulin (pmol/L)
Blood glucose (mmol/L)
Time
2100
2200
2300
0000
0100
0200
0300