Oxytocin improves beta-cell responsivity and glucose tolerance in healthy men

Short title: Oxytocin improves glucose tolerance

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Abstract

In addition to its pivotal role in psychosocial behavior, the hypothalamic neuropeptide oxytocin contributes to metabolic control by suppressing eating behavior. Its involvement in glucose homeostasis is less clear, although pilot experiments suggest that oxytocin improves glucose homeostasis. We assessed the effect of intranasal oxytocin (24 IU) administered to 29 healthy, fasted male subjects on glucose homeostasis measured by means of an oral glucose tolerance test. Parameters of glucose metabolism were analyzed according to the oral minimal model. Oxytocin attenuated the peak excursion of plasma glucose and augmented the early increases in insulin and C-peptide concentrations in response to the glucose challenge, while slightly blunting insulin and C-peptide peaks. Oral minimal model analyses revealed that oxytocin compared to placebo induced a pronounced increase in beta-cell responsivity (PHI_{total}) that was largely due to an enhanced dynamic response (PHI_d), and a more than two-fold improvement in glucose tolerance (disposition index). ACTH, cortisol, glucagon and non-esterified fatty acid concentrations were not or only marginally affected. These results indicate that oxytocin plays a significant role in the acute regulation of glucose metabolism in healthy humans and render the oxytocin system a potential target of antidiabetic treatment.

The hypothalamic neuropeptide oxytocin is released into the circulation via the posterior pituitary and also acts directly on central neuronal receptors. It is primarily known to regulate reproductive and psychosocial functions including attachment, mother-infant bonding and sexual behavior (1; 2). Recent research has moreover indicated that oxytocin contributes to metabolic control by modulating eating behavior and energy expenditure. After subcutaneous administration, oxytocin decreases food intake and increases energy expenditure in rodents (3) and rhesus monkeys (4). Acute inhibitory effects of oxytocin on food intake have likewise been observed in humans (5; 6). These studies have also provided preliminary evidence that oxytocin may exert beneficial effects on glucose homeostasis. In monkeys, four weeks of oxytocin administration reduced plasma glucose concentrations (4). In humans, a single application of oxytocin was shown to blunt the peak plasma glucose response to breakfast intake (5), and to acutely improve insulin sensitivity as indicated by a reduction of fasting insulin concentrations and HOMA-IR (6). These reports are in accordance with earlier studies demonstrating that oxytocin is able to stimulate insulin secretion in animals (7; 8). Intravenous delivery of the peptide was associated with increased insulin secretion during glucose administration also in humans, whereas blood glucose remained unchanged (9). Oxytocin promotes glucose uptake in muscle cells of neonatal rats (10), and the presence of oxytocin receptors in rodent islets of Langerhans suggests an involvement in the release of insulin and glucagon (11). Interestingly, chronic administration of oxytocin was able to improve glucose intolerance, fatty liver, cardiac dysfunction and insulin resistance in animal models of obesity (12-17). Against this background, targeting the oxytocin system has already been advanced as a new therapeutic strategy in the treatment of metabolic disorders (18-20). However, its role in metabolic regulation and glucose homeostasis, particularly in humans, is still largely unexplored (21).

We have systematically investigated the effect of oxytocin on glucose tolerance and beta-cell responsivity in healthy humans. Oxytocin was administered via the intranasal

pathway which enables the transport of peptides from the nasal cavity to the CNS (22) and reliably modulates brain functions in humans (1). Glucose homeostasis was assessed by means of an oral glucose tolerance test (OGTT) and analyzed according to the oral minimal model method.

Research Design and Methods

Subjects. Twenty-nine normal-weight, healthy men participated in the study (age ± SEM, 25.1 ± 0.8 years; BMI, 23.0 ± 0.2 kg/m^2). Body composition assessments by bioelectrical impedance analyses (Nutriguard-M, Data Input, Germany) performed at the start of each experimental session yielded 16.5 ± 0.4 kg body fat and 60.5 ± 0.9 kg lean body mass averaged across conditions (P>0.16 for all comparisons between conditions). All relevant illness as well as abuse of alcohol, nicotine or drugs were excluded by medical history, clinical examination, and routine laboratory tests during screening. All participants gave written informed consent to the study which was approved by the ethics committee of the University of Lübeck and conformed to the principles of the Declaration of Helsinki.

Experimental procedure. Experiments were performed in a double-blind, cross-over, within-subject comparison. Each subject participated in two sessions (oxytocin and placebo) spaced apart at least 14 days with the order of conditions balanced across participants. Subjects were instructed to remain fasted with the exception of drinking water after 2000 h on the day preceding each session.

Following arrival at the lab at 0800 h, a venous cannula was inserted into the subject's nondominant arm to enable blood collection, and blood was sampled at 0840 h and 0855 h for baseline assessments of hormonal parameters, as well as at regular intervals throughout the experiment (cf. Figure 1B). Each blood sampling was accompanied by measurements of heart rate and blood pressure. At 0910 h, six 0.1 ml puffs (three per nostril) of oxytocin

(Syntocinon; Defiante Farmaceutica, Funchal Madeira, Portugal) or placebo (vehicle) were intranasally administered at 30-sec intervals, yielding a total dose of 24 IU oxytocin (0.6 ml) in the verum condition. At 1010 h, i.e., 60 min after spray administration, subjects drank 300 ml Accu-Check Dextrose O.G.T. solution (containing 75 g anhydrous glucose) over a period of 1 min. Energy expenditure was measured by indirect calorimetry using a ventilated-hood system (Deltatrac II, MBM-200 Metabolic Monitor; Datex-Engström Deutschland, Achim, Germany) during baseline and at 1015 h, i.e., immediately after ingestion of the dextrose solution. Subjective ratings of mood and symptoms were frequently assessed throughout the experiment. Mood was assessed with 5-point scales covering the categories good/bad mood, alertness/sleepiness, and calmness/agitation (23). Symptoms were rated on 10-point scales assessing neuroglycopenic symptoms (dizziness, tingling, blurred vision, difficulty in thinking, faintness) and autonomic symptoms (anxiety, palpitation, hunger, sweating, irritability, tremor; (24)).

Analyses of blood parameters. Blood samples for the measurement of glucagon and oxytocin were collected in tubes that contained aprotinine (370 kIU/ml; Roth GmbH, Karlsruhe, Germany). All blood samples were centrifuged and supernatants stored at -80°C. Plasma glucose and lactate were measured in fluoride plasma (Aeroset, Abbott Diagnostics, North Chicago, IL, USA). Routine assays were used to measure insulin, C-peptide, cortisol, adrenocorticotropic hormone (ACTH) (all Immulite, DPC, Los Angeles, CA, USA), glucagon (RIA, IBL International, Hamburg, Germany), and non-esterified fatty acids (NEFAs; ADVIA analyzer, Siemens Healthcare Diagnostics, Eschborn, Germany). Plasma oxytocin concentrations were measured by radioimmunoassay after extraction by the Riagnosis company (Regensburg, Germany).

Oral Minimal Model Method. The oral minimal model method allows the estimation of insulin sensitivity and beta-cell responsivity based on an oral glucose tolerance test. In terms

of reproducibility, the oral indices of insulin sensitivity (S_I) and beta-cell responsivity (PHI) are comparable to those obtained with the intravenous glucose tolerance test (IVGTT) while they are easier to obtain but at the same time more physiological. For calculation purposes, the oral minimal model discerns several subsystems of glucose regulation. For the present analyses, the *Glucose minimal model* and the *C-peptide minimal model* were applied, allowing for the separate estimation of S_I and PHI without confounding influences of the respective other parameter. The *Glucose minimal model* resembles the classic single-compartment IVGTT minimal model but assumes the gastrointestinal tract as a new compartment with the oral glucose dose as the input parameter. It calculates S_I with measured insulin concentrations as input and plasma glucose concentrations as output parameters to be fitted by the model. S_I reflects the ability of insulin to stimulate glucose disposal and inhibit glucose production. The *C-peptide minimal model* estimates beta-cell function in terms of a basal responsivity index ('PHI_{basal}') and a total responsivity index ('PHI_{total}'). It interprets plasma C-peptide concentrations as the output in relation to the observed changes in glucose concentrations as the input parameter. PHI_{total} comprises two distinct components with different interpretations on a cellular level: 'PHI dynamic (PHI_d)', the amount of insulin secreted per unit increase of glucose concentrations during the dynamic phase, which is proportional to the glucose rate of change and is assumed to relate to exocytosis of insulin from secretory vesicles docked to the membrane; and 'PHI static (PHI_s)', which represents insulin release in proportion to plasma glucose levels above a threshold (over basal average static phase secretion per unit over basal average glucose concentration) and is assumed to reflect insulin granule translocation and maturation. The disposition index (DI) is the product of PHI_{total} and S_I and estimates glucose tolerance, i.e., the ability of beta-cells to upregulate insulin secretion in response to a glucose challenge. For a detailed description of the oral minimal model method including respective formulas see reference (25).

Insulin clearance. Fasting insulin clearance was calculated as [C-Peptide in pmol/l at 0840 h]/[insulin in µU/ml at 0840 h]. Insulin clearance during the first two hours of the OGTT was calculated as [AUC C-Peptide in pmol/l (1010-1210 h)]/[AUC insulin in µU/ml (1010-1210 h)].

Statistical Analyses. All data are presented as mean absolute values ± SEM. Analyses were based on analyses of variance (ANOVA) with the within-subject factors "Treatment" (oxytocin vs. placebo) and "Time". Degrees of freedom were corrected using the Greenhouse-Geisser procedure. Significant ANOVA effects were specified by pairwise t-tests and comparisons of areas under the curve (AUC) covering the relevant time periods. For parameters without normal distribution, the Wilcoxon test was used. For blood parameters, baseline adjustment was achieved by subtracting individual baseline values from individual post-intervention measurements. A P-value <0.05 was considered significant.

Results

Glucose homeostasis. Baseline concentrations of oxytocin, glucose, insulin and C-peptide were comparable between conditions (P>0.2 for all comparisons). Intranasal oxytocin administration caused a rapid increase in plasma oxytocin concentrations after 20 min (P<0.001), which remained elevated in the oxytocin condition until 120 min after the glucose test ($F_{1,37}$ = 60.35, P<0.001 for Treatment x Time; Figure 1A). The excursion in plasma glucose concentrations upon the oral glucose load was blunted after oxytocin compared to placebo administration ($F_{3,90}$ = 3.77, P≤0.05 for ANOVA factor Treatment × Time; Figure 1B). This effect was most pronounced in the first 60 min of the OGTT ($F_{2,51}$ = 4.96, P=0.025 for Treatment × Time; P<0.005 for $AUC_{1010\text{-}1110h}$). Serum insulin concentrations showed a more rapid increase in response to the glucose challenge after oxytocin compared to placebo administration 10 min after the glucose load (P<0.04 for pairwise comparison, Wilcoxon test)

and a moderate attenuation of the peak response 60 min thereafter (P=0.058; Figure 1C). The time course of C-peptide concentrations paralleled that of serum insulin, indicating an immediate elevation 10 min after glucose ingestion (P<0.03) followed by a blunted peak response after oxytocin compared to placebo administration (P<0.02; Figure 1D). Supplementary analyses indicated that the rapid oxytocin-induced increase in insulin concentrations was statistically unrelated to the respective decrease in plasma glucose $AUC_{1010\text{-}1110h}$ values (r=0.162; P=0.4, Pearson's coefficient).

In the oral minimal model analyses, glucose tolerance was strongly enhanced by oxytocin in comparison to placebo as reflected by a more than twofold increase in DI (257.8 ± 83.7 vs. 110.7 ± 34.4, P=0.001; Figure 1E). Oxytocin also induced a trendwise increase in the ability of insulin to stimulate glucose disposal and inhibit glucose production (S_I, 4.0 ± 1.0 vs. 2.5 ± 0.6, P=0.059). As a marker of beta-cell responsivity, PHI_{total} was augmented in the oxytocin compared to the placebo condition (34.3 ± 2.2 vs. 23.0 ± 1.8, P<0.001; Figure 1F). This effect was primarily mediated by an oxytocin-induced increase in the dynamic component PHI_d (659 ± 50 vs. 598 ± 69, P=0.039; Figure 1G), which indicates enhanced dynamic-phase insulin secretion. PHI_{basal} (4.8 ± 0.2 vs. 4.6 ± 0.2, P>0.4) and PHI_s (78.7 ± 7.8 vs. 77.5 ± 10.4, P>0.4) did not differ between conditions, indicating that oxytocin did not affect basal and static-phase insulin secretion.

Exploratory analyses of insulin clearance did not reveal any significant differences between the oxytocin and the placebo conditions, neither for fasting insulin clearance (101.4 ± 5.6 vs. 107.3 ± 6.5, P>0.1) nor for insulin clearance in the first two hours of the OGTT (58.5 ± 3.0 vs. 58.3 ± 3.3, P>0.9).

Counterregulatory hormones, lactate and NEFA. Baseline concentrations of ACTH, cortisol, glucagon and lactate did not differ between conditions (all P>0.2). Across the experimental period, circulating concentrations of ACTH (Figure 2A) and cortisol (Figure 2B) were

likewise comparable between conditions (P>0.5 for Treatment × Time). Oxytocin treatment increased glucagon concentrations from 60 min after the start of the OGTT on (Figure 2C); this effect was however not corroborated by analyses covering the whole experimental period ($F_{2,67}$ = 2.28, P=0.1 for Treatment × Time) or by ANOVA and AUC analyses focusing on the time period between 60 and 180 min after the OGTT (all P>0.1). The differences in glucagon concentrations between conditions at 60 and 180 min post-OGTT were statistically unrelated to the oxytocin-induced blunting of the glucose response ($AUC_{1010\text{-}1110h}$; r=-0.153, P=0.4; r=0.031, P=0.874, respectively; Pearson's coefficients).

Lactate concentrations were not affected by oxytocin administration ($F_{3,96}$ = 1, P>0.3 for Treatment × Time). NEFA concentrations displayed a trend towards lower baseline NEFA concentrations in the oxytocin compared to the placebo condition (P=0.058), but no differential effects of oxytocin during the experimental period ($F_{2,64}$ = 2, P≥0.1 for Treatment × Time). The oxytocin-induced blunting of the peak glucose response was still evident when including the slight baseline difference in NEFA concentrations as a covariate in the respective analyses ($F_{1,27}$ = 7.31, P<0.02 for ANCOVA factor Treatment).

Energy expenditure, heart rate, blood pressure and psychological parameters. Energy expenditure assessed by indirect calorimetry was comparable between the placebo and the oxytocin condition during baseline (1307±36 vs. 1317±39 kcal/d, P>0.7) as well as after glucose intake (1427±44 vs. 1465±48 kcal/d, P>0.1). Heart rate and diastolic blood pressure did not differ between conditions throughout the experiment (all P>0.4). Systolic blood pressure was higher in the oxytocin compared to the placebo condition during the final assessment, i.e., 180 min after the OGTT (129 vs. 122 mmHg, P=0.005), but otherwise comparable (P>0.1 for Treatment × Time and single time-point comparisons). Mood and subjective well-being according to the self-rated categories good/bad mood (P>0.2),

alertness/sleepiness (P>0.2) and calmness/agitation (P>0.3), as well awareness of neuroglycopenic (P>0.1) and autonomic symptoms (P>0.2) were not affected by oxytocin.

Discussion

We provide evidence for an acutely enhancing effect of oxytocin administration on glucose tolerance and beta-cell responsivity in healthy men. We found that in fasted subjects submitted to an oral glucose tolerance test after intranasal oxytocin delivery, the neuropeptide attenuates the glucose peak response to a glucose challenge. This effect was accompanied by an increased early response of insulin and C-peptide in conjunction with a dampened peak response of these hormones. The improving effect of oxytocin on glucose metabolism was further investigated by in-depth data analyses according to the oral minimal model, which demonstrated that oxytocin significantly increased PHI_{total} as a marker of beta-cell responsivity. In particular, oxytocin boosted the dynamic response to glucose as represented by PHI_d, which suggests an improved ability of beta-cells to secrete insulin in response to a rise in glucose concentrations. This enhancing effect on beta-cell function was moreover confirmed by the more than two-fold increase in insulin-dependent glucose tolerance (DI) in the oxytocin compared to the placebo condition. Oxytocin also slightly enhanced insulin sensitivity (S_I), suggesting a stronger effect of insulin on stimulating glucose disposal and inhibiting glucose production after oxytocin in comparison to placebo administration.

Our results of an acute stimulatory effect of oxytocin on beta-cell function and glucose tolerance are supported by previous experiments showing beneficial oxytocin effects on glucose homeostasis (5; 6). Lawson and coworkers observed a reduction in insulin concentrations and HOMA-IR values after oxytocin vs. placebo administration in combination with ad-libitum meal intake (6). Previous data from our group demonstrated an oxytocin-mediated attenuation of the peak glucose response to breakfast intake (5). Using a standardized methodological approach based on the oral minimal model, the present study

systematically reveals oxytocin's contribution to glucose homeostasis in humans. Experiments in animals focusing on the sequelae of obesity have shown that insulin resistance, glucose intolerance, pancreatic islet hypertrophy and hepatic steatosis can be ameliorated by oxytocin administration (15; 26; 27). In accordance, patients with diabetes mellitus type 2 and obese subjects appear to display reduced serum oxytocin levels (28). Interestingly, anti-diabetic effects of oxytocin have been found to emerge prior to decreases in body weight (17), suggesting that long-term glucoregulatory effects of the peptide are not a consequence of weight loss. This assumption is in line with our results of acute beneficial effects of oxytocin on glucose metabolism in healthy normal-weight men.

The mechanisms behind the effect of oxytocin on glucose homeostasis are still in need of investigation. Oxytocin neurons in the supraoptic nucleus of the hypothalamus have been found to serve as glucose sensors (29). It is however unclear whether central or peripheral mechanisms prevail in oxytocin's role in glucose homeostasis (8; 30), not least because after experimental peripheral administration, oxytocin can stimulate its own central release via feed-forward mechanisms (18). As evidenced by immunohistochemical methods, the islets of Langerhans harbor oxytocin receptors (11). In primary cultures of neonatal rat cardiomyocytes, oxytocin augments glucose uptake by stimulating the release of intracellular calcium and activating phosphoinositide-3-kinase, calcium-calmodulin kinase kinase, and AMP-activated protein kinase (10; 31). The impact of oxytocin on insulin secretion may thus be mediated both centrally, i.e., via vagal cholinergic neurons innervating beta cells, as well as peripherally, i.e., by triggering phosphoinositide and protein kinase-C activation in beta cells (32). Oxytocin has also been shown to reduce gluco- and lipotoxicity and to modulate the regulation of cytokines like adiponectin and leptin (12; 13). Further studies in isolated pancreatic islets are clearly needed to elucidate the relative contributions of these and related pathways to oxytocin's glucoregulatory action. With the paradigm of intranasal oxytocin administration as chosen in the present experiments, such a distinction is not possible.

Intranasally administered neuropeptides reach the central nervous compartment (22) and elicit functional effects (19), as has been repeatedly observed also for oxytocin (1; 5). Still, the rapid and sustained increase in circulating oxytocin after its intranasal delivery suggests that oxytocin might exert at least some of the observed effects via a direct, peripheral action on pancreatic beta cells, which may in particular hold for the increase in first-phase insulin release.

Against the background of the known interaction between the oxytocin and other endocrine signaling systems (20), hormonal factors may act as mediators of oxytocin's glucoregulatory effect. The increase in first-phase insulin concentrations after oxytocin administration, which was followed by a slight decrease of peak insulin responses 50 min later, is in accordance with reports of a stimulatory impact of oxytocin on insulin secretion in animals and humans (7-9; 17). First-phase insulin release is assumed to be of particular relevance for glucose tolerance inasmuch as defects in this mechanism might precede subsequent diabetic changes (33). In our study in healthy, normal-weight men, first-phase insulin release however was statistically unrelated to the ensuing attenuation of the glucose peak response. We did not employ tracers and were therefore not able to directly measure glucose fluxes, which leaves open the question whether the enhancement of glucose tolerance was primarily mediated by improved glucose uptake or to an even larger extent by a reduced glucose production or enhanced glucose effectiveness. Circulating concentrations of NEFA are important regulators of insulin sensitivity and glucose disposal (34; 35). We did not observe effects of oxytocin on NEFA concentrations, and the glucoregulatory effect of the peptide was still evident when analyses were corrected for small baseline differences in NEFA levels, which argues against a direct contribution of fatty acid metabolism to our findings.

Since in agreement with other studies (36), the time courses of the counterregulatory hormones ACTH and cortisol were comparable between conditions, it is unlikely that they

were involved in the mediation of oxytocin's effects on glucose tolerance, although a link between HPA axis activity and glucose homeostasis clearly exists. We have previously observed a dampening influence of oxytocin on HPA axis activity under basal conditions (5), but this effect appears to be even more pronounced in response to social and physical stress (37; 38). While in principle it is conceivable that in the present study, subtle changes in ACTH or cortisol concentrations before the OGTT challenge elapsed our attention, there are also hints that oxytocin's influence on HPA axis activity is of a rather chronic nature (39). Weak signs of a stimulatory effect of oxytocin on glucagon concentrations – which has been observed in previous experiments (7; 8; 40-42) – appeared delayed and were statistically unrelated to the blunted peak glucose response. At the moment, there is only a small number of reports on oxytocin's role in human glucose homeostasis, and they moreover exhibit marked differences in study protocols that concern oxytocin doses and application routes as well as the glycemic state of the subjects. Therefore, the role of oxytocin in endocrine glucoregulatory networks clearly should be at the focus of further research efforts. They should also include subjects of different ethnicity and female subjects, also because of oxytocin's strong implication in reproductive function.

In summary, our data obtained in a systematic experimental approach using the oral minimal model demonstrate that oxytocin acutely improves beta-cell responsivity and glucose tolerance in healthy men. In conjunction with the promising oxytocin-induced improvements of obesity-related diabetic phenotypes observed in other studies (14-16) and the favorable side-effect profile of this neuropeptide (43), our findings support the idea of testing oxytocin in a clinical context. Here, the peptide might be particularly effective in reducing postprandial hyperglycemia, a major pathological feature of diabetes mellitus type 2. Clearly, oxytocin's potential to improve metabolic control across the range of normal and impaired glucose tolerance is yet to be fully discovered.

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No potential conflicts of interest relevant to this article were reported.

J.K., V.O. and M.H. designed the study and wrote the manuscript. J.K., V.O. and K.R. enrolled subjects and carried out experiments for the study. J.K., V.O., K.R., F.P., C.C. and M.H. analyzed the data. J.K., V.O., S.B., F.P., C.C., H.L. and M.H. discussed the results. K.R., S.B., F.P., C.C. and H.L. contributed to writing the manuscript. J.K. and M.H. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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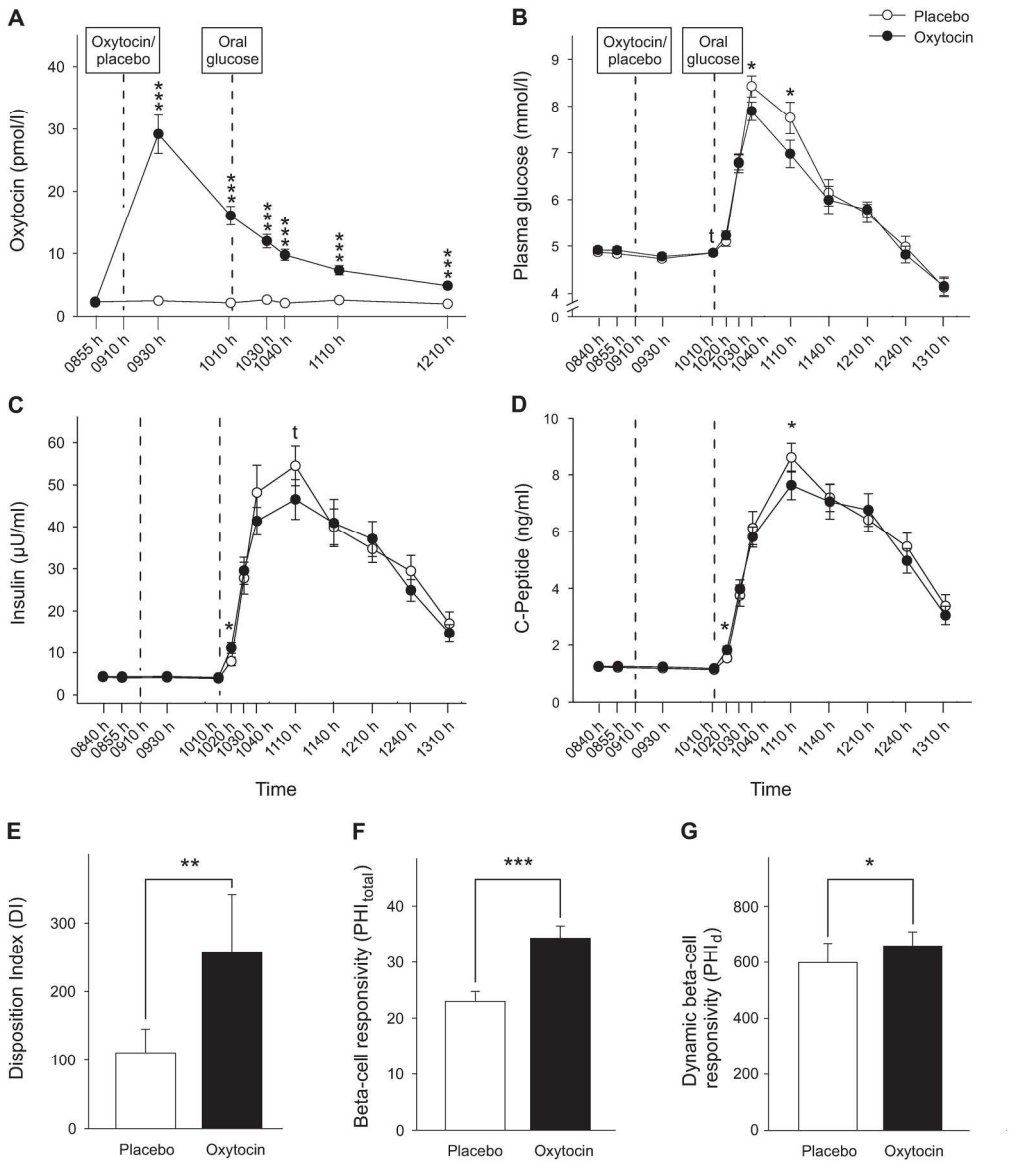
Figure legends

Figure 1: Circulating oxytocin concentrations and parameters of glucose homeostasis. Mean ± SEM plasma or serum concentrations of oxytocin (A), glucose (B), insulin (C) and C-peptide (D) at baseline and after administration of 24 IU oxytocin (black circles) or placebo (white circles). Oxytocin or placebo was administered at 0910 h and the OGTT solution (containing 75 g glucose) was consumed one hour later at 1010 h. Bottom panels depict oral minimal model-derived estimates of the disposition index (DI; E) and beta-cell responsivity (PHI_{total}; F; Phi_d; G) in the oxytocin (black bars) and placebo condition (white bars). $^{t}P \leq 0.1$, $*P \leq 0.05$, $**P < 0.01$, $***P < 0.001$ as derived from paired t-tests or Wilcoxon tests; N=29.

Figure 2: Counterregulatory hormones. Mean ± SEM plasma or serum concentrations of ACTH (A), cortisol (B) and glucagon (C) at baseline and after administration of 24 IU oxytocin (black circles) or placebo (white circles). Oxytocin or placebo was administered at 0910 h and the OGTT solution (containing 75 g glucose) was consumed one hour later at 1010 h. $^{t}P \leq 0.1$, $*P \leq 0.05$ as derived from paired t-tests; N=29.



Circulating oxytocin concentrations and parameters of glucose homeostasis.
Figure 1
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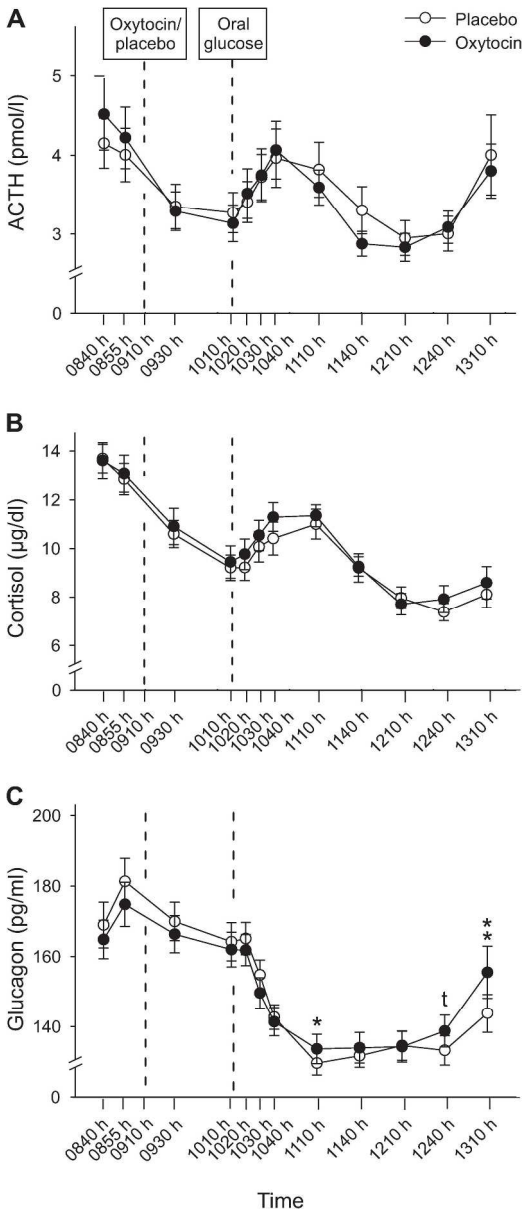

Counterregulatory hormones.
Figure 2
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