Safety of intranasal human insulin: a review

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Abstract

Subheadings – aims

The nasal application of human insulin is frequently used for investigating brain insulin action. It is utilized in studies on the physiological role of insulin in the human brain as well as in therapeutic interventional trials and its effects have been investigated after both acute and long-term administration. This review aimed to assess the safety of intranasal human insulin in human studies and the temporal stability of nasal insulin sprays.

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Material and Methods

The electronic search was performed using MEDLINE. We selected original research on intranasal human insulin without further additives in humans.

The studies included could be of any design as long as they used human intranasal insulin as their study product. All outcomes and adverse side effects were excerpted.

Results

Thirty-eight studies on 1092 persons with acute application and 18 studies on 832 persons with treatment lasting between 21 days and 9.7 years were identified. No cases of symptomatic hypoglycemia or severe adverse events were reported. Transient local side effects in the nasal area were frequently experienced after intranasal insulin and placebo spray, while other adverse events were less commonly reported. There are no reports of subjects who were excluded due to adverse events.

No instances of temporal stability of nasal insulin were reported in the literature. Tests on insulin that had been repacked into spray flasks revealed that it had a chemical stability of up to 57 days.

Conclusions

. Our retrospective review of published studies on intranasal insulin did not reveal any safety concerns. There is, however, insufficient data to ensure long-term safety of this modality of chronic insulin administration. Improved insulin preparations that cause less nasal irritation would be desirable for future treatment.

Introduction

Research in animals clearly demonstrates that the brain is an insulin-sensitive organ (1–3). Over the past few years, several studies have shown that the human brain is also insulin responsive (4,5). However, much of the experimental technology applied in animals obviously cannot be used in humans, since it is either too invasive or relies on genetic manipulation. One technique – the administration of insulin in the form of a nasal spray - has, nevertheless, proved to be particularly useful in studying insulin action in the human brain (6). Within minutes of spray application, the peptide hormone is detectable in the lumbar cerebrospinal fluid (CSF) in humans for at least 80 minutes (7). The pharmacokinetics of nasal human insulin spray were recently studied in 8-week-old mice (8). Following nasal application, labeled human insulin entered the brain within minutes and was detectable for more than 60 minutes in all investigated brain areas. Less than 3% of the administered insulin entered the circulation and no peripheral metabolic effects were detected up to 24 h post spray application. This study suggests that the transport of insulin into the brain after intranasal administration involves different mechanisms than the one found at the blood-brain barrier and may therefore enable to study the effects on the brain independent of the blood-brain barrier.

Early reports of the nasal administration using 40 U insulin in humans found no significant changes of circulating insulin levels (7,9), suggesting that intranasally applied insulin does not pass into the circulation. However, subsequent studies with higher insulin doses clearly demonstrated that small amounts of the nasally administered peptide pass into the bloodstream (10). Importantly, the amount of insulin absorbed is so small that no major changes in blood glucose were detected in doses of up to 160 U nasal insulin (4). A recent study on different doses of nasal insulin proposed that 160 U might be necessary to introduce acute profound effects on both the central and the peripheral nervous system (10).

A variety of additives have been tested to enhance the absorption of intranasally applied insulin into the bloodstream to facilitate glucose lowering effects for the treatment of diabetes

(11,12). However, this is beyond the scope of the present review which focuses on the application of regular human insulin.

Using the intranasal administration of human insulin, as well as other methods to stimulate brain insulin action, a number of human brain areas were characterized to respond to the peptide. This led to functional consequences on memory, mood, eating behavior, and body weight, as well as metabolism in the entire organism (for recent review of functions of brain insulin in humans, please see (4,5)). These studies also illustrate that responses to insulin application in the brain are not uniform. A substantial number of persons were found to be brain insulin-resistant. This condition was, furthermore, linked to a number of potential behavioral and metabolic consequences (for recent reviews, please see (4,5)).

Besides addressing physiologic and pathophysiologic aspects of brain insulin action in humans, several studies used intranasal insulin with a therapeutic intention:

Nasal insulin has been administered to children with high risk for type 1 diabetes (13–16). This disease is caused by an autoimmune destruction of the insulin-producing pancreatic beta cells. Nasal insulin was applied to determine whether this treatment induces sufficient immune tolerance to prevent onset of disease. Up to now, however, there are no reports that the onset of the disease is positively influenced by nasal insulin.

In patients with the rare genetic Phelan-McDermid syndrome, nasal insulin administration was observed to have beneficial effects on cognition and social skills (17,18).

In addition to these trials, which enrolled mainly children, a number of studies were conducted in adults: Nasal insulin was also used in trials to improve cognitive function in patients with mild cognitive impairments or in early stages of Alzheimer's disease (19–25). Since positive effects on cognition and related biomarkers were reported, follow-up trials are under way (e.g., clinical trials.gov NCT01767909).

Nasal insulin administration acutely reduced cigarette craving in smokers (26). Intranasal insulin also improved olfaction in a small-scale trial on patients with an impaired sense of

smell (27). in trials on patients with schizophrenia (28–30), bipolar disorder (31), or major depression (32). However, it did not show any effect on disease control.

Despite the use of intranasal insulin in many clinical studies in humans, only a limited number of these trials systematically reported safety aspects. Furthermore, the safety of intranasal insulin application has not yet been reviewed in the literature. From the evidence available on possible side effects and adverse reactions to the intranasal application of human insulin, we therefore include a detailed overview of the safety aspects of this technique. We also provide data on the temporal stability of human insulin after repacking into spray flasks.

Methods

Data source

We carried out a broad search of the available English-language literature. The electronic search was performed using MEDLINE. Articles published between 1999 and 2017 were included, with the cutoff date November 2nd, 2017. Prior to 1999, no clinical data on the impact of intranasal insulin and its side effects was provided in the literature. The following search terms were used: intranasal insulin, nasally insulin, nasal insulin. Bibliographies of current reviews on brain insulin were also analyzed (2,4–6,33,34).

Literature Screening

Study selection was performed on two levels of study screening (see Fig. 1). First, abstracts were reviewed for the following exclusion criteria: publication of abstracts only, comments, and reviews, animal or in vitro studies, and languages other than English. Although intranasal insulin has been tested in a number of studies in animals (n=33), this is beyond the scope of the current review.

Full articles were then obtained for all manuscripts accepted on the first level. For level two screening, inclusion required that the studies contained at least one of the following pieces of information: insulin dose, insulin product, study population. If a manuscript provided lacked information on safety, we requested information on this issue from the authors.

Although the studies included could be of any design, human intranasal insulin as the study product was a prerequisite. All outcomes and adverse side effects were excerpted. In studies reporting adverse side effects, we endeavored to determine both the number of patients evaluated and the evaluation of chronic or acute application as well as the nature of any side effects in the placebo or the verum group. All articles were screened for effects on blood glucose and the appearance of hypoglycemia. In many studies, however, there was no mention of the the occurrence of adverse side effects. In addition, we included the main

outcome for all manuscripts. Multiple publications describing the same cohorts of patients were identified and entered into the catalog only once to avoid duplcounting.

Stability

We tested the stability of the insulin product Actrapid, since this was the human insulin product used in most of the studies. Insulin content of the spray solutions was assessed by high-performance liquid chromatography (HPLC) according to recommendations of the FDA and the European Pharmacopoeia (Ph. Eur.). USP Insulin Human RS (USP, Rockville, MD, United States) was used as a reference standard. Three batches (8 spray flasks each) of Actrapid insulin 100 U/ml (Novo Nordisk, Bagsvaerd, Denmark) were prepared by transferring 6 ml of Actrapid insulin solution into sterile 10-ml screw cap bottles under aseptic conditions. Each bottle was then closed with a sterile screw cap. Four flasks from each batch were stored at 2-8°C, while the others four were stored at room temperature (<25°C). At days 0, 14, 28 and 57, flasks were analyzed after diluting an aliquot in 0.01N hydrochloric acid.

Measurements in triplicate were performed on a modular HPLC system (Shimadzu, Kyoto, Japan) equipped with an octadecyl silica gel column (Luna-Omega-C 18, 250 mm x 4.6 mm, 5 µm particle size, Phenomenex, Aschaffenburg, Germany) interfaced to a photo diode array detector (SPD-M20A) measuring at 214 nm. The column oven temperature was maintained at 40°C, and the injection volume was 10 µL with a mobile phase flow of 1 ml/min. Chromatograms were analyzed by LCsolution (Shimadzu, Kyoto, Japan).

As recommended in the monography “insulin human” of the European Pharmacopoeia, the resolution of HPLC peaks of human insulin and porcine insulin was tested with a resulting resolution of 1.24. Capacity factors, separation efficiencies and tailing factors were within the limits recommended by the FDA.

Results

A flow diagram outlining the systematic review process is provided in Fig. 1. The initial literature research identified 409 articles for screening, 35 of which were excluded since the full text article was not available. Of the 374 articles reviewed, 309 did not meet inclusion criteria. Altogether, 65 articles were therefore suitable for analysis.

The results of 38 studies with acute administration of intranasal insulin are summarized in Table 1. A total of 1092 persons participated in these studies; nasal insulin doses ranged between 10 U and 160 U. No cases of hypoglycemia and no severe adverse events were reported. Adverse events and main outcomes are summarized in Table 1.

The results of 18 studies with chronic administration of intranasal insulin are summarized in Table 2. These studies included 832 persons with treatment duration of between 21 days and 9.7 years. Daily nasal insulin doses ranged between 20 U and 160 U or 0.5 – 1.5 U/kg body weight. One case of symptomatic hypoglycemia was reported during treatment with placebo spray (35). No cases of severe hypoglycemia were recorded.

While no severe side effects were reported in any of the included studies, most manuscripts contained information on other adverse effects. These are summarized in Table 2 and aggregated by category in Table 3.

Stability results

None of the analyzed manuscripts contained any information on the stability of nasal insulin preparations. We therefore tested the temporal stability of insulin after repacking into nasal spray flasks under two storage conditions: at room temperature (<25°C) and under refrigeration (2-8°C). No visible changes were observed. Mean recovery rates were 98.8% (day 28) and 97.1% (day 57) of the initial concentration under refrigeration and 100.4% (day 28) and 98.1% (day 57) of the initial concentration at room temperature respectively (Fig. 2).

Inspection of the HPLC profiles revealed no accumulation of insulin degradation products over time.

Discussion

Only half of the 38 identified publications on acute effects of intranasal human insulin contained information on possible side effects. The majority of the manuscripts reporting the chronic application of nasal insulin also provided information on possible side effects. Fortunately, upon personal contact, authors of most of the remaining publications provided us with this information. Besides systematically aggregating this safety information, we provide measurements on the stability of human insulin after repacking into spray flasks for up to 3 months.

The majority of the identified studies used human insulin manufactured by Novo Nordisc (product name Actrapid or Novolin R). Some used insulin manufactured by Eli Lilly (product name Humulin R) or Sanofi (product names Insuman Rapid and H-Insulin 100). All these products contain similar recombinant human insulin and comparable excipients. While the dosage of excipients differs slightly between the products, none are believed to be in a range that cause harm when applied to the nose (36).

Safety

Since more than 1904 persons in the analyzed literature experienced no severe adverse events, intranasal insulin application appears to be relatively safe. Only one trial was specifically designed to test the safety of nasal administration of human insulin (35). It included 20 adults, who received 60 U human insulin and placebo in a double-blind randomized crossover design for 3 weeks (per arm). Insulin effects on the nose were addressed by an otorhinolaryngologist who performed a rhinoscopy, assessed mucociliary clearance, nasal airway patency, and nasal airflow resistance. No objective signs of acute adverse effects or functional disturbances in the nose were established. However, modest

subjective irritation was commonly reported by the participants. No changes in insulin antibody concentrations were detected.

One major safety concern of insulin administration is the risk of hypoglycemia. Indeed, a number of publications reported absorption of small amounts of the nasally applied peptide hormone into the bloodstream (37–42). Our review of the literature revealed that intranasal application of human insulin caused a decrease in blood glucose of between 0.2 mmol/l (4 mg/dl) and 0.5 mmol/l (9 mg/dl) in some studies (26,38,41–48). This decline in blood glucose persisted much longer than the temporary rise in circulating insulin post insulin nasal spray. Hence, this is presumably not only due to the spillover of nasal insulin into circulation, but also to enhanced peripheral insulin sensitivity caused by insulin action in the human brain (37,39,40). No cases of symptomatic or severe hypoglycemia after nasal insulin were reported.

Even if not reported in most publications, our own experience and personal communication with other scientists revealed that many subjects experience a short-term (< 5min) burning sensation in the nose directly after spray application. In one case, this persisted for about 24 h (19). Since this burning sensation was also frequently reported after placebo spray, it is probably caused by the excipients and not by the actual human insulin. Furthermore, one case of nose bleed after acute administration of 40 U nasal insulin has been reported so far (19). There are no reports of participants who prematurely discontinued a study due to side effects.

In a study in longtime smokers who had to abstain from smoking before spray application (26), a number of additional side effects were reported (see Table 1). To date, these particular side effects following acute application of nasal insulin have never been reported elsewhere, suggesting that nicotine withdrawal played a role here.

No further side effects were reported in the 1092 persons studied after acute administration of nasal insulin.

As in acute application, chronic treatment resulted in local side effects in the nasal area. A number of infections occurred during chronic treatment with nasal insulin. However, frequencies were comparable between insulin and placebo, and since infections are common in the patient populations studied, it is unlikely that nasal insulin alters the immune system in a way that predisposes to infectious diseases. A number of other side effects were reported less frequently, none of which were considered severe by the respective investigators. Taken together, the intranasal application of insulin appears to be relatively safe in both an acute and a chronic setting. All reported side effects were not severe and no subjects were reported to have been excluded due to adverse events. However, local side effects of long-term exposure of the nasal mucosa to insulin have not yet been extensively studied and future studies are therefore necessary to address this issue.

Stability

Our measurements revealed the chemical stability of human insulin after repacking it into spray flasks for up to 57 days. In line with earlier investigations (49), HPLC analysis revealed that the container system did not significantly influence the stability of human insulin solution.

Perspective – challenges for clinical use

As nasal insulin is relatively safe and stable after repacking in spray flasks, it is an appropriate tool to assess brain insulin effects in humans. The insulin formulations currently available can be used in acute and chronic clinical trials. However, nasal application often induces local effects such as a burning sensation in the nose. Improved insulin preparations for nasal use would therefore be advantageous to future studies and possible therapeutic application in patients.

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Conflict of interest

None of the authors has any conflict of interest of direct relevance to the contents of this study.

Figure Legend

Figure 1
Flow diagram of the literature search

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Table 1 – Major outcomes and safety details of studies with acute application intranasal human insulin

Studies on acute effects of nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Kern et al., 1999 (50)	120 U over 90 minutes versus Placebo (cross-over)	18 volunteers	H-Insulin 100 Sanofi; Sanofi	Changes in auditory evoked potentials, no effect on plasma insulin, glucose or plasma norepinephrine levels	No information	none
Born et al., 2002 (51)	40 U once versus placebo (randomized parallel)	8 healthy subjects (insulin arm), 5 healthy subjects (placebo arm)	No information	Increase in CSF insulin, no effect on serum insulin	No information	No information
Reger et al., 2006 (19)	20 or 40 U or placebo once (cross-over)	26 memory-impaired subject and 35 normal controls	Novolin R, Novo Nordisk	Improved verbal memory, no effect on plasma insulin or blood glucose	Nose bleed (1 subject at 40 U), nose soreness for about 24h (treatment not specified)	none
Benedict et al., 2008 (44)	160 U versus placebo once (cross-over)	32 normal-weight subjects (18 females)	Actrapid, Novo Nordisk	Decreased food intake in men, but not in women, improved memory in women, but not in men	Decrease in blood glucose (by about 0.24 mmol/l)	none
Bohringer et al., 2008 (52)	40 U versus placebo once (randomized parallel)	26 healthy young males	Actrapid, Novo Nordisk	Diminished cortisol response to the Trier social stress test	none	none
Mark A. Reger et al., 2008 (53)	10, 20, 40, 60 U versus placebo once (cross-over)	59 controls and 33 memory-impaired patients	Novolin R, Novo Nordisk	Improved verbal memory, peak effect with 20 U no effect on plasma insulin or blood glucose	Personal communication: Some cases of rhinitis, no other side effects.	none
Guthoff et al., 2010 (54)	160 U versus placebo once (cross-over)	9 healthy subjects (5 males)	Actrapid, Novo Nordisk	Altered processing of food pictures (assessed by fMRI)	Personal communication: burning sensation in the nose in some participants (no more than 5 minutes); no other AEs	none

Studies on acute effects of nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Krug et al., 2010 (55)	160 U versus placebo once (cross-over)	14 healthy postmenopausal women	Actrapid, Novo Nordisk	No effect on food intake, enhanced memory	Personal communication: tingling or mild burning sensation in the nose in some participants; no other AEs	none
Stingl et al., 2010 (56)	160 U once versus. placebo (cross-over)	10 lean/10 overweight healthy volunteers	Actrapid, Novo Nordisk	Changes in resting state dynamics (assessed by MEG)	Personal communication: burning sensation in the nose in some participants (no more than 5 minutes); no other AEs	none
Benedict et al., 2011 (57)	160 U versus Placebo once (cross-over)	19 healthy men	Actrapid, Novo Nordisk	Enhanced postprandial thermogenesis, lower postprandial circulating insulin and C-peptide levels	Personal communication: tingling or mild burning sensation in the nose in some participants; no other AEs	none
Fan et al., 2011 (28)	40 U versus placebo once (randomized parallel)	30 patients with schizophrenia	Humulin, Eli Lilly	No effect on verbal memory or sustained attention	No effect on plasma glucose	none
Guthoff et al., 2011 (58)	160 U versus placebo once (cross-over)	10 lean and 10 obese subject	Actrapid, Novo Nordisk	Altered cerebral processing of food pictures in lean, but not obese (assessed by MEG)	Personal communication: burning sensation in the nose in some participants (no more than 5 minutes); no other AEs	none
Stockhorst et al., 2011 (48)	20 U once (randomized parallel)	32 healthy young subjects	Insuman Rapid, Sanofi	Conditioned increase in peripheral insulin	Slight decline in blood glucose, no adverse side effects	none

Studies on acute effects of nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Stein et al., 2011 (21)	60 U (n=16) versus placebo (n=16) 4 times daily for 2 days (total 480 U insulin) (parallel-design)	32 patients with mild to moderate Alzheimer´s disease (16 females treated with nasal insulin)	Humulin, Eli Lilly	No effect of nasal insulin on memory	Personal communication: no side effects	Personal communication: No hypogylcemia was measured
Grichisch et al., 2012 (59)	160 U versus oral caffeine (200mg) once (cross-over)	8 healthy subjects (3 males)	Actrapid, Novo Nordisk	No effect on global cerebral blood flow, i.e. no direct vasodilatatory effect of nasal insulin	Personal communication: burning sensation in the nose in some participants (no more than 5 minutes); no other AEs	Personal communication: none
Hallschmid et al., 2012 (45)	160 U versus placebo once (randomized parallel)	30 healthy women	Actrapid, Novo Nordisk	Decreased postprandial appetite, decreased postprandial intake of chocolate cookies	Slight decrease in plasma glucose	none
M Heni et al., 2012; Ketterer et al., 2014 (43,60)	160 U versus Placebo once (cross-over)	103 volunteers	Actrapid, Novo Nordisk	Change in HOMA-IR and change in regular brain activity, no effect on cortisol levels	Decline in blood glucose by about 0.2 mmol/l, burning sensation in the nose in some participants (no more than 5 minutes)	none
Jauch-Chara et al., 2012 (61)	40 U versus placebo once (cross-over)	15 healthy men	Actrapid, Novo Nordisk	Increased brain ATP and phosphocreatine levels	No effect on blood glucose (measured every 5 minutes), no other side effects reported	none
Brünner et al., 2013 (47)	40 U once versus placebo (cross-over)	14 healthy subjects (7 females)	Actrapid, Novo Nordisk	Decrease in olfactory threshold	Slight decline in blood glucose (about 0.2 mmol/l)	none

Studies on acute effects of nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Kullmann et al., 2013 (62)	160 U once versus placebo (cross-over)	17 female volunteers	Actrapid, Novo Nordisk	Modification of reward processes and prefrontal brain activity, assessed by fMRI	Personal communication: burning sensation in the nose in some participants (no more than 5 minutes); no other AEs	none
Heni et al., 2014 (40)	160 U once versus placebo during systemic hyperinsulinemia (cross-over)	10 lean and 5 obese healthy men	Actrapid, Novo Nordisk	Improved peripheral insulin sensitivity, modulation of hypothalamic activity (assessed by fMRI), change in heart rate variability	Personal communication: burning sensation in the nose in some participants (no more than 5 minutes); no other AEs	none
Iwen et al., 2014) (63)	160 U once versus placebo (cross-over)	14 healthy men	Actrapid, Novo Nordisk	Decrease in circulating free fatty acids and lipolysis	Personal communication: tingling or mildly burning sensation in the nose in some participants; no other AEs	none
Novak et al., 2014; Zhang et al., 2015 (64,65)	40 U once versus saline (randomized parallel)	15 patients with type 2 diabetes, 14 controls	Novolin R, Novo Nordisk	Improvement in cognitive function, change in cerebral blood flow (assessed by MRI)	No serious adverse events, no nasal irritation, no allergic reactions	none
Ferreira de Sá et al., 2014; Schilling et al., 2014 (66,67)	40 U insulin (n=13), 30mg Cortisol (n=12), 30mg Cortisol + 40 U insulin (n=15), placebo (n=14) (parallel-design)	54 healthy volunteers	Actrapid, Novo Nordisk	No effect of insulin on processing of food cues	Personal communication: tingling or mildly burning sensation in the nose in some participants; no other AEs	Personal communication: none
Brünner et al., 2015 (68)	40U once versus placebo (cross-over)	18 male subjects	Actrapid, Novo Nordisk	Improved, delayed but not immediate odor-cued recall of spatial memory	None	none

Studies on acute effects of nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Gancheva et al., 2015 (38)	160 U once versus placebo (cross-over)	10 patients with type 2 diabetes (1 female), 10 healthy volunteers (3 females)	Actrapid, Novo Nordisk	Improvement in hepatic energy metabolism and decline in liver fat content in lean subjects	Slight decline in blood glucose	none
Kullmann et al., 2015, 2017b, 2017a (69–71)	160 U once versus placebo (cross-over)	25 lean (10 female) and 23 (11 female) overweight healthy volunteers	Actrapid, Novo Nordisk	Change in regional brain activity (assessed by fMRI)	Personal communication: burning sensation in the nose in some participants (no more than 5 minutes); no other AEs	none
Schöpf et al., 2015 (27)	40 U insulin (n=10), NaCl at later time point (n=7) once	10 patients with smell loss	Actrapid, Novo Nordisk	Improved olfactory sensitivity and intensity	Personal communication: No adverse events	Personal communication: none
Feld et al., 2016 (41)	160 U versus placebo (cross-over)	16 healthy men and 16 healthy women	Actrapid, Novo Nordisk	Increased growth hormone concentrations in the night-half following nasal insulin, impaired memory encoding on subsequent day	Temporary, slight decline in blood glucose	none
Brünner et al., 2016 (72)	40 U versus placebo once (cross-over)	16 healthy men	Actrapid, Novo Nordisk	No effect of nasal insulin on declarative memory or hippocampal activity	none	none

Studies on acute effects of nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Hamidovic et al., 2017 (26)	60 U versus placebo once	19 healthy smokers (cross-over) and 37 healthy smokers (parallel) abstained from smoking for 36h	Novolin R, Novo Nordisk	Reduction in nicotine craving, Increase in circulating cortisol during psychosocial stress	- Slight decrease in blood glucose - Rinorrhea (Placebo: N=1; Insulin: N=1) - Nasal irritation (Placebo: N=5, Insulin N=7) - Dizziness (Placebo: N=0, Insulin N=1) - Sweating (Placebo: N=0, Insulin N=2) - Headache (Placebo: N=0, Insulin N=1) - Confusion (Placebo: N=1, Insulin N=2) - Tingling in mouth (Placebo: N=0, Insulin N=1) - Shaking (Placebo: N=0, Insulin N=1) - Anxiety (Placebo: N=1, Insulin N=1) - Restlessness (Placebo: N=0, Insulin N=1) - Discomfort (Placebo: N=0, Insulin N=1) - Watering eyes (Placebo: N=0, Insulin N=2)	none

Studies on acute effects of nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Heni et al., 2017 (39)	160 U versus placebo once during systemic hyperinsulinemia (cross-over)	11 lean and 10 overweight healthy men	Actrapid, Novo Nordisk	Improvement in peripheral insulin sensitivity by suppression of endogenous glucose production and stimulation of glucose uptake into tissue. Change in regional brain activity in hypothalamus and striatum, detailed characterization and mimicking of spillover of nasal insulin into circulation	Personal communication: burning sensation in the nose in some participants (no more than 5 minutes); no other AEs	none
Rodriguez-Raecke et al., 2017 (73)	40 U versus placebo once (cross-over)	24 healthy males	Actrapid, Novo Nordisk	Improved gustatory sensitivity	none	none
Santiago and Hallschmid, 2017 (46)	160 U versus placebo once (cross-over)	51 healthy (32 young and 19 elderly) men and women	Actrapid, Novo Nordisk	Reduced food intake, no effect on sleep patterns	Slight decline in blood glucose (around 0.48 mmol/l), no other side effects reported	none
Akintola et al., 2017 (74)	40 U versus placebo (cross-over)	19 adults (11 older and 8 young)	Actrapid, Novo Nordisk	Improved brain perfusion in occipital lobe and thalamus in older persons	No effect on blood glucose. No other side effects reported	none
Thienel et al., 2017 (42)	160 U versus placebo once (cross-over)	14 elderly and 30 young healthy subjects	Actrapid, Novo Nordisk	Reduced cortisol levels in the night half following nasal insulin in elderly, but not in young participants	Temporary slight decline in blood glucose	none

Studies on acute effects of nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Opstal et al., 2017 (75)	40 U versus placebo (cross-over)	8 healthy, normal weight adult male	Actrapid, Novo Nordisk	Enhanced effect of oral glucose ingestion on the hypothalamus	Personal communication: One participant with runny nose after insulin and placebo spray, no other side effects	none
Kullmann et al., 2017 (10)	40 U, 80 U, 160 U versus placebo (four-way cross-over)	9 healthy men	Actrapid, Novo Nordisk	Dose-depended effect on regional brain activity and on the autonomic nervous system	Personal communication: burning sensation in the nose in some participants (no more than 5 minutes); no other AEs	none
		1092 persons acutely treated with nasal insulin				

Table 2 – Major outcomes and safety details of studies with chronic application intranasal human insulin

Studies on effects of chronic nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Kupila et al., 2003 (35)	60 U once daily over 3 weeks versus Placebo (cross-over)	20 non-diabetic adults (11 females)	Actrapid, Novo Nordisk	No effect on blood glucose	No nasal adverse effects (rhinoscopy and further tests before and after treatment), 11 subjects with nasal stinging or unpleasant odor, no induction of diabetes-related auto-antibodies	one symptomatic hypoglycemia (during placebo treatment)
Benedict et al., 2004 (76)	4x40 U/d for 8 weeks or placebo (randomized parallel)	38 healthy volunteers (24 males) (age 18 to 34 years)	Actrapid, Novo Nordisk	Improved declarative memory, reduced anger, enhanced self-confidence, lower serum cortisol in insulin group	Personal communication: tingling or mildly burning sensation after application in the nose in some participants; some subjects' partners complained about odor; no other AEs.	none
Hallschmid et al., 2004 (77)	4x40 U/d for 8 weeks or placebo (randomized parallel)	40 healthy subjects (24 males)	Actrapid, Novo Nordisk	Reduction of body weight in men, body weight gain in women	No effect on glucose and insulin levels. Personal communication: tingling or mildly burning sensation after application in the nose in some participants; some subjects' partners complained about odor; no other AEs.	none
Harrison et al., 2004 (13)	40 U daily versus placebo for the first 10 days, thereafter 2 consecutive days weekly for 6 month (randomized parallel)	38 children with one or more auto-antibodies	Humulin, Eli Lilly	Induction on immune changes consistence with mucosal tolerance to insulin	No local or systemic adverse events	none

Studies on effects of chronic nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Benedict et al., 2005 (78)	Acute study: 20 U every 10 min over 2h (240 U) versus placebo (cross-over) Chronic study: 4x40 U/d for 8 weeks or placebo (randomized parallel)	8 young men (acute study), 8 men and 8 women (chronic study)	Actrapid, Novo Nordisk	Acute, but not chronic rise in blood pressure, no effect on heart rate	No information	none
Benedict et al., 2007 (79)	4x40 U/d human insulin or placebo or 4x40 U/d insulin aspart for 8 weeks (randomized parallel)	36 young, healthy, lean men	Actrapid, Novo Nordisk	Enhanced insulin induced memory improvement after intranasal insulin aspart	No effect on glucose. Personal communication: tingling or mildly burning sensation after application in the nose in some participants; some subjects' partners complained about odor; no other AEs.	none
Hallschmid et al., 2008 (80)	4x40 U/d for 8 weeks or placebo (randomized parallel)	30 obese men	Actrapid, Novo Nordisk	Improvement of declarative memory and mood, no effect on body weight, reduction of ACTH and cortisol	Personal communication: tingling or mildly burning sensation after application in the nose in some participants; some subjects' partners complained about odor; no other AEs.	none

Studies on effects of chronic nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Haavisto et al., 2010; Näntö-Salonen et al., 2008; Ryhänen et al., 2011 (14,16,81)	1 U/kg versus placebo once daily, median duration 1.8 years (range 0-9.7 years) (randomized parallel)	224 infants positive for 2 or more auto-antibodies and 40 siblings	Actrapid, Novo Nordisk	No effect on the progression of type 1 diabetes, no increase in auto-antibodies during treatment	Nasal irritation (75% insulin group and 72% placebo group), symptomatic time per treatment month longer in the placebo group, no difference in nasal mucosa in a subgroup, nasal discharge 83% insulin group and 86% placebo group), cough 75% insulin group and 77% placebo group) and fever (75% insulin group and 87% placebo group), middle ear (and sinus infections 32% insulin group and 42% placebo group), gastrointestinal symptoms 60% insulin group and 64% placebo group), no difference in more rare adverse events	No hypoglycemia

Studies on effects of chronic nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
M. A. Reger et al., 2008 (20)	20 U twice daily versus placebo for 21 days (randomized parallel)	25 cognitive-impaired subjects	Novolin R, Novo Nordisk	Improved verbal memory, improved attention, increased Aß40/42 ratio, no effect on insulin and blood glucose	- No serious adverse events, - Headache (1 subject on insulin), - nasal dripping (2 subject on placebo, 1 subject on insulin), - weakness (1 subject on insulin), - sneezing (1 subject on placebo)	Blood glucose between 60 and 70 mg/dL (1 insulin)
Schmidt et al., 2009 (17)	0.5-1.5 U/kg/d for one year, no placebo group	6 children with Phelan-McDermid syndrome (age 16 months to 9.5 years)	Actrapid, Novo Nordisk	Improvement in motor development, cognitive function and spontaneous activity	- changes in balance, extreme sensitivity to touch and general loss of interest (one patient), - intermittent nose bleeding (one patient) - no effect on HbA1c, cortisol or insulin auto-antibodies	none
Fourlanos et al., 2011 (82)	40 U daily versus placebo for the first 10 days, thereafter 2 consecutive days weekly for 12 months (randomized parallel)	52 adults with recent onset type 2 diabetes	Humulin, Eli Lilly	Blunted insulin anti-body response, no effect on metabolism	Personal communication: no side effects	Personal communication: No hypogylcemia was measured

Studies on effects of chronic nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
McIntyre et al., 2012 (31)	40 U daily versus placebo for 8 weeks (randomized parallel)	62 adults with bipolar I/II disorder	Novolin R, Novo Nordisk	Improvement of executive function	Insulin group: intranasal irritation (13.6%), anxienty (4.9%), nose bleed (2.9%). Placebo group: nasal irritation (21%), increased appetite (3.6%), light-headedness (2.4%)	none
Claxton et al., 2013; Craft et al., 2012 (23,24)	20 or 40 U or placebo daily for 4 months (randomized parallel)	104 cognitive-impaired patients	Novolin R, Novo Nordisk	Delayed progression of cognitive decline and PET assessed brain dysfunction	- No severe adverse events - lightheadedness and/or dizziness (Placebo: 10%, 20 U: 8%, 40 U: 13%) - headache (Placebo: 3%, 20 U: 8%, 40 U: 5%) - nose bleed (Placebo: 0%, 20 U: 8%, 40 U: 3%) - rhinitis (Placebo: 3%, 20 U: 17%, 40 U: 8%) - upper respiratory tract infections (Placebo: 7%, 20 U: 6%, 40 U: 3%) - Fall (Placebo: 7%, 20 U: 3%, 40 U: 3%) - Rush (Placebo: 7%, 20 U: 3%, 40 U: 3%) All these side effects in ≤ 5 participants per group	none

Studies on effects of chronic nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Fan et al., 2013; Li et al., 2013 (29) (30)	4x40 U/d (N=21) versus placebo (N=24) for 8 weeks (parallel-design)	45 patients with schizophrenia	Humulin, Eli Lilly	No improvement in schizophrenia symptoms or cognition. No effect on body weight, glucose or lipids.	No serious adverse events - Wheezing (insulin 10%; placebo 0%) - Coughing (insulin 10%;placebo 0%) - Trouble breathing (insulin 5%; placebo 0%) - Nasal congestion (insulin 10%; placebo 4%) - Hypersalivation (insulin 5%; placebo 0%) - Nausea (insulin 5%; placebo 0%) - Vomiting (insulin 5%; placebo 0%) - Numbness (insulin 5%; placebo 0%) - Poor concentration (insulin 10%; placebo 4%) - Confusion (insulin 5%; placebo 0%) - Insomnia (insulin 19%; placebo 0%) - Drowsiness (insulin 10%; placebo 0%) No patient withdrew from study on account of AEs	none

Studies on effects of chronic nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
Zwanenburg et al., 2016 (18)	age 1-3 years 20 U/d, age 3-9 years 30 U/d, age 9-18 years 40 U/d for 18 months or placebo (stepped wedge design)	25 patients with Phelan-McDermid syndrome (1-16 years)	Humulin, Eli Lilly	No statistically significant improvement in cognitive function, significant effect on cognition and social skills and patients > 3 years	- No severe adverse events, - Transitory nasal irritation, - Mild pancreatitis (1 child under placebo after more than 5 months' treatment), - Nosebleeds and irritation of nasal area for 1 or 2 days (12 patients)	Not measured
Cha et al., 2017 (32)	4x40 U/d versus placebo for 4 weeks (cross-over)	35 patients with major depressive disorder	Humulin, Eli Lilly	No effect on mood	Personal communication: Local irritation in the nose, no other side effects.	Personal communication: none
Craft et al., 2017 (83)	2x20 U/d (n=12) versus insulin detemir (n=12) versus placebo (n=12) for 4 months (parallel-design)	36 patients with mild cognitive impairment or Alzheimer´s disease	Humulin, Eli Lilly	Better memory	No serious adverse events. Rhinitis and upper respiratory symptoms (most common). No further information available.	No information
Scherer et al., 2017 (84)	4x40 U/d (n=10) versus placebo (n=10) for 4 weeks (parallel-design)	20 healthy males	Actrapid, Novo Nordisk	No effect on body weight or intrahepatic lipids. Reduction of circulating branched-chain amino acids.	Mild burning sensation in the nose	none

Studies on effects of chronic nasal application of human insulin						
Author	**Dose**	**Study population**	**Insulin product**	**Main outcome**	**Adverse side effects**	**Hypoglycemia**
		832 persons treated with nasal insulin in studies with multiple administrations				

Table 3 – Overview about reported side effects of chronic application of nasal insulin in humans

	Näntö-Salonen et al., 2008 (81)	Fan et al., 2013 (29)	M. A. Reger et al., 2008 (20)	Craft et al., 2012 (24)	McIntyre et al., 2012 (31)	Zwanenburg et al., 2016 (18)	Schmidt et al., 2009 (17)	Craft et al., 2017 (83)
	264 children N=91 in each group were analyzed for AEs	45 patients with schizophrenia	25 cognitive-impaired patients	104 cognitive-impaired patients (N=30 with placebo, N=30 with 20 U and N=38 with 40 U)	62 adults with bipolar I/II disorder	25 children with Phelan-McDermid syndrome	6 children with Phelan-McDermid syndrome	
Nasal irritation / sneezing	75% (N=68) insulin group and 72% (N=66) placebo group	N=2 insulin group and N=0 placebo group	N=1 insulin group and N=0 placebo group	-	13.6% insulin group and 21% placebo group	N=12	-	
Nasal discharge / rhinitis	83% (N=76) insulin group and 86% (N=78) placebo group	-	N=2 insulin group and N=1 placebo group	Placebo: 3% (N=1), 20U: 17% (N=8), 40 U: 8% (N=4)	-	-	-	N=5 insulin group and N=2 placebo group
Nasal congestions	-	N=2 insulin group and N=1 placebo group	-	-	-	-	-	
Nose bleed	-	-	-	Placebo: 0%, 20U: 8% (N=6), 40 U: 3% (N=3)	2.9% insulin group	N=12	N=1	
Cough	75% (N=68) insulin group and 77% (N=70) placebo group	N=2 insulin group and N=0 placebo group	-	-	-	-	-	
Wheezing	-	N=2 insulin group and N=0 placebo group	-	-	-	-	-	

	Näntö-Salonen et al., 2008 (81)	Fan et al., 2013 (29)	M. A. Reger et al., 2008 (20)	Craft et al., 2012 (24)	McIntyre et al., 2012 (31)	Zwanenburg et al., 2016 (18)	Schmidt et al., 2009 (17)	Craft et al., 2017 (83)
Hypersalivation	-	N=1 insulin group and N=0 placebo group	-	-	-	-	-	
Fever	75% (N=68) insulin group and 87% (N=80) placebo group	-	-	-	-	-	-	
Middle ear and sinus infections	32% (N=30) insulin group and 42% (N=39) placebo group	-	-	-	-	-	-	
Upper respiratory tract infections	-	-	-	Placebo: 7%, 20U: 6%, 40 U: 3%	-	Frequency not specified	-	
Gastrointestinal symptoms	60% (N=56) insulin group and 64% (N=59) placebo group	N=2 insulin group and N=0 placebo group	-	-	-	Frequency not specified	-	N=1 insulin group and N=1 placebo group
Numbness	-	N=1 insulin group and N=0 placebo group	-	-	-	-	-	
Poor concentration/ confusion	-	N=3 insulin group and N=1 placebo group	-	-	-	-	-	
Insomnia	-	N=4 insulin group and N=0 placebo group	-	-	-	-	-	
Anxiety	-	-	-	-	4.9% insulin group	-	-	-

	Näntö-Salonen et al., 2008 (81)	Fan et al., 2013 (29)	M. A. Reger et al., 2008 (20)	Craft et al., 2012 (24)	McIntyre et al., 2012 (31)	Zwanenburg et al., 2016 (18)	Schmidt et al., 2009 (17)	Craft et al., 2017 (83)
Drowsiness / weakness	-	N=2 insulin group and N=0 placebo group	N=1 insulin group and N=0 placebo group	Placebo: 10%, 20 U: 8%, 40 U: 13%	2.4% placebo group	-	-	N=1 insulin group and N=0 placebo group
Fall	-	-	-	Placebo: 7%, 20 U: 3%, 40 U: 3%	-	-	-	
Headache	-	-	N=1 insulin group and N=0 placebo group	Placebo: 3%, 20 U: 8%, 40 U: 5%	N=1 insulin group	-	-	N=2 insulin group and N=0 placebo group
Rush	-	-	-	Placebo: 7%, 20 U: 3%, 40 U: 3%	-	-	-	
Changes in balance, extreme sensitivity to touch and general loss of interest	-	-	-	-	-	-	N=1	
Musculoskeletal injury/pain	-	-	-	-	-	-	-	N=1 insulin group and N=0 placebo group

Fig. 1