**The Effect of Intranasal Insulin on Appetite and Mood in Women with and without Obesity: An Experimental Medicine Study**

1. Aim

This experimental medicine study aimed to investigate the homeostatic, reward, and cognitive mechanisms that underlie the effects of intranasal insulin on appetite in women with and without obesity. It was hypothesised that intranasal insulin would reduce appetite and improve cognition in women with and without obesity.

1. Participants

Participants were lean women (18.5-25 kg/m2) and women with obesity (≤30 kg/m2). Participants were recruited from the University of Birmingham and the surrounding community through posters and social media.

1. Design

A randomised, crossover, double-blind, placebo-controlled design was used. All participants received 160 IU/1.6 mL of intranasal insulin or 160 IU/1.6 mL of placebo in a counterbalanced order at least one week apart.

1. Materials
   1. Demographic information: BMI, age (years), ethnicity, haemoglobin A1c (hbA1c)
   2. Beck Depression Inventory – II (BDI-II): The BDI is a 21-item scale measuring depression severity (Beck et al., 1996).
   3. The Dutch Eating Behaviour Questionnaire (DEBQ): The DEBQ is a 33-item self-report questionnaire comprised of three subscales: ‘Emotional Eating’, ‘External Eating’, and ‘Dietary Restraint’ (van Strien et al., 1986).
   4. The Power of Food Scale (PFS): The PFS is a 15-item scale that measures the appetite for palatable foods at three levels of proximity (Food Available, Food Present, and Food Tasted) to yield a total score of appetite for palatable foods (Lowe et al., 2009).
   5. Visual Analogue Scales (VAS): VAS assess current appetite, mood, and physical state. The participants rated how they felt at that moment in relation to 14 sensations (alertness, drowsiness, happiness, hunger, fullness, desire to eat, thirst, disgust, anxiety, sadness, and withdrawn, lightheaded, nausea, faint) by placing a vertical mark through a 10cm horizontal line with left and right anchors indicating the extremes of each sensation (‘completely absent’ to ‘most I could imagine’). Completed questionnaires were then measured from the left end of each horizontal line to the place where the vertical mark was drawn for each question. VAS were analysed using the factor structure: ‘Arousal’ (alertness, drowsiness, and happiness), ‘Appetite’ (hunger, fullness, and desire to eat), ‘Negative Effects’ (disgust, anxiety, sadness, and withdrawn), ‘Physical Effects’ (lightheaded, nausea, and faint). Thirst was treated as a separate factor. Pre- and post-dose VAS results were converted to area under the curve (AUC) values using the trapezoid method.
   6. Positive and Negative Affect Schedule (PANAS): The PANAS is a 20-item scale that measures positive and negative affect (Watson et al., 1988). The resulting PANAS factors are Positive Affect (PA) and Negative Affect (NA). Pre- and post-dose PANAS results were converted to area under the curve (AUC) values using the trapezoid method.
   7. Cookie intake measured in grams. Cookie liking ratings (0-100 mm) collected at the beginning and end of the snack. Cookie eating rate measured as grams per minute.
   8. Delay discounting: The monetary discounting task included nine delays ranging from one day to one year. On a white screen, participants saw the question ‘Which would you prefer?’, with two choices: £xx now or £xx after a delay (varying from one day to one year) and were asked to select the preferred option. A similar paradigm was used for food, with questions consisting of food variables instead of money. Participants were able to select their favourite food from a bank of food images that included sweet and savoury energy dense palatable foods. Questions required a choice between a smaller amount of food now and a larger amount later, for example ‘Which would you prefer?’ with the options ‘one bite of chocolate now or a bar of chocolate in a month?’. Users selected their choice using the left and right arrow keys on a keyboard. Data are expressed as area under the curve where a value closer to 1.00 indicates a preference for delayed rewards.
   9. Emotional Test Battery (see www.p1vital.com) is a computerised battery consisting of validated emotional cognitive tasks (Thomas et al., 2016) that have been used in previous single-dosing drug experiments (Harmer et al., 2003; Harmer et al., 2009). The following three tasks from the ETB were included:
      1. Emotional Categorisation Task (ECAT): Sixty positive and negative adjectives (e.g., cheerful, hostile) were presented for 500ms. Participants responded with a button box to indicate whether they would like or dislike to be described as such. Words were matched for meaningfulness, length, and frequency of occurrence. Accuracy (percentage) and reaction times (RT) (ms) by valence were measured.
      2. Emotional Recall Task (EREC): Participants were given four minutes to recall as many words from the ECAT task as could be remembered within a 4-minute period. Task instructions and the timer were presented on the computer, and the participant wrote recalled words on paper. Accuracy for correctly recalling words presented in the ECAT were measured as items remembered by valence. Recalling words that were not presented in the ECAT were labelled as commission errors and were recorded as items incorrectly recalled by valence.
      3. Emotional recognition memory task (EMEM): Participants were presented with 60 personality descriptor words derived from the ECAT, along with 60 matching novel distractor words. Participants were instructed to indicate whether the word had been presented during the ECAT using a dedicated button box. Percentage accuracy for correctly recognising words that appeared in the ECAT, and RT for correct responses were recorded by valence. Commission errors (items) for incorrectly classifying a distractor word as having appeared in the ECAT was recorded as percentage incorrectly recognised by valence.
      4. Verbal paired associated: Participants were instructed to memorise 60 associated word pairs that were presented for 2 seconds on a computer screen presented using E-Prime 2.0 software (Psychology Software Tools, Pittsburgh, PA). Following word pair presentation, participants received a cue word on the computer screen and responded aloud with the target word to be scored for accuracy by the researcher. After the participant responded, the participant pressed the spacebar to reveal the target word on the screen. Participants were tested again an hour later as a measure of delayed recall. Total amount of words recalled out of 60 was recorded.
      5. N-Back: To measure working memory capacity, a visuospatial n-back task (Kirchner, 1958) was presented via E-Prime 2.0 (Psychology Software Tools, Pittsburgh, PA) software. Blue circles were presented on a white 3x3 grid for 500ms. Participants were instructed to indicate if the circle was in the same position (‘1’ on the keyboard) or a different position (‘2’ on the keyboard) as it was two (2-back) and three trials back (3-back). Accuracy (proportion) and RT (ms) by stimuli (2 and 3-back) were recorded.
      6. Picture Rating Task Recall: Participants performed a food and non-food picture rating task in a magnetic resonance imaging (MRI) scanner. Participants viewed a range (36 each category) of low- and high-calorie food (equally distributed in sweet and savoury) and non-food items (visually matched) and rated each image for liking. At the end of the test day, participants were asked to recall as many of the images as possible from the picture rating task and to record these responses on paper. Accuracy (percentage) by category (food and non-food) was recorded.
      7. All blood samples were collected via an intravenous catheter. Four millilitres of blood were collected on the first test day prior to drug administration for the determination of glycated haemoglobin (HbA1C; mmol/mol) reflecting baseline insulin sensitivity. On both test days, four blood samples (4mL) for the determination of insulin and glucose were collected at baseline, five minutes post-drug administration, 135 minutes post-drug administration, and 155 minutes post-drug administration. Capillary blood glucose was measured throughout the day as a safety precaution to monitor hypoglycaemia.
2. Procedure

Participants arrived at the Wellcome Trust Clinical Research Facility (University Hospitals Birmingham NHS Foundation Trust – Queen Elizabeth Hospital) to confirm eligibility via a screening session. Participants completed the following questionnaires: DEBQ, PFS, and the SCID. Height, weight, and body fat were measured. The participant then had a medical check with a trained medical doctor that consisted of a pregnancy test, blood pressure, electrocardiogram (ECG), and a verbal medical history.

Participants arrived at the Wellcome Trust Clinical Research Facility at 10:30, 11:00, or 12:00. Participants were instructed to eat breakfast as normal prior to the study. Upon arrival, participants completed their first VAS and PANAS and then received a medical check that consisted of documentation of medical changes since the screening appointment, a pregnancy test, and a baseline blood draw for the determination of HbA1C, insulin, and glucose. Following confirmation of negative pregnancy results, participants consumed lunch. After lunch, participants completed a set of the VAS/PANAS and then self-administered the IN insulin under the guidance of the researcher and a nurse. Participants were instructed to inhale 16 0.1 mL puffs (eight per nostril) of insulin and placebo respectively at 30-second intervals, amounting to a total dose of 1.6 mL insulin or placebo. Five minutes post-drug administration, a blood draw was taken and then another set of the VAS/PANAS was completed. Participants then underwent an fMRI scan for 1.5 hours. During the scan, participants completed an inhibition task and a picture rating task. Following the fMRI scan, participants completed the DD task. Participants then completed another set of the VAS/PANAS and had another blood draw (135 minutes post-dose). The participants then consumed ad libitum chocolate cookies to measure hedonic eating. The final (155 minutes post-dose) blood draw was taken following the snack and the participants completed another set of VAS/PANAS. The participants then completed the immediate recall phase of the VPA task. Following the VPA, participants completed the ECAT, N-back, EREC, and EMEM followed by a set of VAS/PANAS. The delayed recall phase of the VPA was then completed. Next, the participants were given five minutes to recall as many of the images that were presented during the food rating task completed in the scanner and to record these responses on paper. The participants then completed a final set of VAS/PANAS and a blood glucose safety check before concluding the test day

1. Explanation of the SPSS data file:

Note: Data cells filled with 99999 indicate missing data. Data cells filled with 111111 indicate excluded data removed on the basis of statistical outliers and chance responding.

List and explanation of all the variables in order of SPSS columns. All reaction time data recorded in milliseconds.

1. ID
2. BMI\_status: 1 = lean, 2 = obese
3. Age (years)
4. BMI
5. Ethnicity: 1=white; 2=mixed; 3=Asian/Asian British; 4=Black; 5=Arab; 6=Hispanic
6. HbA1C (mmol/mol)
7. BDI\_Total = total Beck Depression Inventory score
8. DEBQ\_RestraintEating = score on the Restraint scale of the Dutch Eating Behaviour Questionnaire
9. DEBQ\_ExternalEating = score on the External Eating scale of the Dutch Eating Behaviour Questionnaire
10. DEBQ\_EmotionEating = score on the Emotional Eating scale of the Dutch Eating Behaviour Questionnaire
11. PFS\_Total = total score on the Power of Food Scale
12. Insulin\_GLUCOSE\_predose = pre dose glucose (mmol/L) recording in the insulin condition
13. Insulin\_GLUCOSE\_5mins\_postdose = 5 minutes post dose glucose recording in the insulin condition
14. Insulin\_GLUCOSE\_135mins\_postdose = 135 minutes post dose glucose recording in the insulin condition
15. Insulin\_GLUCOSE\_155mins\_postdose = 155 minutes post dose glucose recording in the insulin condition
16. Insulin\_GLUCOSE\_final = 300 minutes post dose glucose recording in the insulin condition
17. Placebo\_GLUCOSE\_predose = pre dose glucose recording in the placebo condition
18. Placebo\_GLUCOSE\_5mins\_postdose = 5 minutes post dose glucose recording in the placebo condition
19. Placebo\_GLUCOSE\_135mins\_postdose = 135 minutes post dose glucose recording in the placebo condition
20. Placebo\_GLUCOSE\_155mins\_postdose = 155 minutes post dose glucose recording in the placebo condition
21. Placebo\_GLUCOSE\_final = 300 minutes post dose glucose recording in the placebo condition
22. INSULIN\_predose\_insulin = pre dose insulin value (mIU/L) in the insulin condition
23. INSULIN\_5mins\_postdose\_Insulin = 5 minutes post dose insulin value in the insulin condition
24. INSULIN\_135minspostdose\_Insulin = 135 minutes post dose insulin value in the insulin condition
25. INSULIN\_155mins\_postdose\_Insulin = 155 minutes post dose insulin value in the insulin condition
26. INSULIN\_predose\_placebo = pre dose insulin value in the placebo condition
27. Placebo\_5mins\_postdose\_insulin = 5 minutes post dose insulin value in the placebo condition
28. Placebo\_135mins\_postdose\_insulin = 135 minutes post dose insulin value in the placebo condition
29. Placebo\_155mins\_postdose\_insulin = 155 minutes post dose insulin value in the placebo condition
30. Insulin\_Cookie\_intake\_grams = cookie intake in the insulin condition
31. Placebo\_Cookie\_intake\_grams = cookie intake in the placebo condition
32. Insulin\_Cookie\_liking\_first = initial liking rating (mm) for the cookie snack in the insulin condition
33. Insulin\_cookie\_liking\_last = final cookie rating for the cookie snack in the insulin condition
34. Placebo\_cookie\_liking\_first = initial cookie rating for the cookie snack in the placebo condition
35. Placebo\_cookie\_liking\_last = final cookie rating for the cookie snack in the placebo condition
36. Insulin\_Cookie\_EatingRate = cookie eating rate (g/m) in the insulin condition
37. Placebo\_Cookie\_EatingRate = cookie eating rate in the placebo condition
38. Insulin\_VPA\_Immediate = total items correct out of 60 possible on the Verbal Paired Associates (VPA) task for the immediate phase in the insulin condition
39. Insulin\_VPA\_Delayed = total items correct out of 60 possible on the VPA task for the delayed phase in the insulin condition
40. Placebo\_VPA\_Immediate = total items correct out of 60 possible on the VPA task for the immediate phase in the placebo condition
41. Placebo\_VPA\_Delayed = total items correct out of 60 possible on the VPA task for the delayed phase in the placebo condition
42. Insulin\_N\_back\_3\_Back\_Accuracy = percentage accuracy for 3-back on the n-back task in the insulin condition
43. Insulin\_N\_back\_2\_Back\_Accuracy = percentage accuracy for 2-back on the n-back task in the insulin condition
44. Insulin\_N\_back\_3\_back\_RT = reaction time for correct responses on the 3-back of the n-back task in the insulin condition
45. Insulin\_N\_back\_2\_Back\_RT = reaction time for correct responses on the 2-back of the n-back task in the insulin condition
46. Placebo\_N\_back\_3\_Back\_Accuracy = percentage accuracy for 3-back on the n-back task in the placebo condition
47. Placebo\_N\_Back\_2\_back\_Accuracy = percentage accuracy for 2-back on the n-back task in the placebo condition
48. Placebo\_N\_back\_3\_Back\_RT = reaction time for correct responses on the 3-back of the n-back task in the placebo condition
49. Placebo\_N\_back\_2\_Back\_RT = reaction time for correct responses on the 2-back of the n-back task in the placebo condition
50. Insulin\_DD\_Money\_AUC = area under the curve (AUC) value for money on the delay discounting task in the insulin condition
51. Insulin\_DD\_Food\_AUC = AUC value for food on the delay discounting task in the insulin condition
52. Placebo\_DD\_Money\_AUC = AUC value for money on the delay discounting task in the placebo condition
53. Placebo\_DD\_Food\_AUC = AUC value for food on the delay discounting task in the placebo condition
54. Insulin\_ECAT\_ACC\_POS = percentage accuracy for positive valence words on the Emotional Categorisation Task (ECAT) in the insulin condition
55. Insulin\_ECAT\_ACC\_NEG = percentage accuracy for negative valence words on the ECAT in the insulin condition
56. Insulin\_ECAT\_RT\_POS = reaction time for correct responses for positive valence words on the ECAT task in the insulin condition
57. Insulin\_ECAT\_RT\_NEG = reaction time for correct responses for negative valence words on the ECAT task in the insulin condition
58. Insulin\_EREC\_ACC\_POS = correctly recalled items for positive valence words on the Emotional Recall Task (EREC) in the insulin condition
59. Insulin\_EREC\_ACC\_NEG = correctly recalled items for negative valence words on the EREC in the insulin condition
60. Insulin\_EREC\_FA\_POS = incorrectly recalled items for positive valence words on the EREC in the insulin condition
61. Insulin\_EREC\_FA\_NEG = incorrectly recalled items for negative valence words on the EREC in the insulin condition
62. Insulin\_EMEM\_ACC\_POS = percentage accuracy for positive valence words on the Emotional Recognition Memory Task (EMEM) in the insulin condition
63. Insulin\_EMEM\_ACC\_NEG = percentage accuracy for negative valence words on the EMEM in the insulin condition
64. Insulin\_EMEM\_FA\_POS = percentage incorrect for positive valence words on the EMEM in the insulin condition
65. Insulin\_EMEM\_FA\_NEG = percentage incorrect for negative valence words on the EMEM in the insulin condition
66. Insulin\_EMEM\_RT\_POS = reaction time for correct responses for positive valence words on the EMEM in the insulin condition
67. Insulin\_EMEM\_RT\_NEG = reaction time for correct responses for negative valence words on the EME in the insulin condition
68. Placebo\_ECAT\_ACC\_POS = percentage accuracy for positive valence words on the Emotional Categorisation Task (ECAT) in the placebo condition
69. Placebo\_ECAT\_ACC\_NEG = percentage accuracy for negative valence words on the ECAT in the placebo condition
70. Placebo\_ECAT\_RT\_POS = reaction time for correct responses for positive valence words on the ECAT task in the placebo condition
71. Placebo\_ECAT\_RT\_NEG = reaction time for correct responses for negative valence words on the ECAT task in the placebo condition
72. Placebo\_EREC\_ACC\_POS = correctly recalled items for positive valence words on the Emotional Recall Task (EREC) in the placebo condition
73. Placebo\_EREC\_ACC\_NEG = correctly recalled items for negative valence words on the Emotional Recall Task (EREC) in the placebo condition
74. Placebo\_EREC\_FA\_POS = percentage incorrect for positive valence words on the EMEM in the placebo condition
75. Placebo\_EREC\_FA\_NEG = percentage incorrect for negative valence words on the EMEM in the placebo condition
76. Placebo\_EMEM\_ACC\_POS = percentage accuracy for positive valence words on the Emotional Recognition Memory Task (EMEM) in the placebo condition
77. Placebo\_EMEM\_ACC\_NEG = percentage accuracy for negative valence words on the EMEM in the placebo condition
78. Placebo\_EMEM\_FA\_POS = percentage incorrect for positive valence words on the EMEM in the placebo condition
79. Placebo\_EMEM\_FA\_NEG = percentage incorrect for negative valence words on the EMEM in the placebo condition
80. Placebo\_EMEM\_RT\_POS = reaction time for correct responses for positive valence words on the EMEM in the placebo condition
81. Placebo\_EMEM\_RT\_NEG = reaction time for correct responses for negative valence words on the EMEM in the placebo condition
82. Insulin\_PRTRecall\_TotalFood\_Percent = percentage of correct food images recalled from the Picture Rating Task (PRT) in the insulin condition
83. Insulin\_PRTRecall\_Object\_Percent = percentage of correct object images recalled from the PRT in the insulin condition
84. Placebo\_PRTRecall\_TotalFood\_Percent = percentage of correct food images recalled from the PRT in the placebo condition
85. Placebo\_PRTRecall\_Object\_Percent = percentage of correct object images recalled from the PRT in the placebo condition
86. PA\_AUC\_Predose\_Insulin = pre dose positive affect ratings (area under the curve (AUC)) on the Positive and Negative Affect Schedule (PANAS) in the insulin condition
87. NA\_AUC\_Predose\_Insulin = pre dose negative affect ratings (AUC) on the PANAS in the insulin condition
88. PA\_AUC\_Predose\_Placebo = pre dose positive affect ratings (AUC) on the PANAS in the placebo condition
89. NA\_AUC\_Predose\_Placebo = pre dose negative affect ratings (AUC) on the PANAS in the placebo condition
90. AUC\_PostDose\_Insulin\_PA = post dose positive affect ratings (AUC) on the PANAS in the insulin condition
91. AUC\_PostDose\_Insulin\_NA = post dose negative affect ratings (AUC) on the PANAS in the insulin condition
92. AUC\_PostDose\_Placebo\_PA = post dose positive affect ratings (AUC) on the PANAS in the placebo condition
93. AUC\_PostDose\_Placebo\_NA = post dose negative affect ratings (AUC) on the PANAS in the placebo condition
94. Thirst\_AUC\_Predose\_Insulin = pre dose thirst ratings (AUC) on the visual analogue scale (VAS) in the insulin condition
95. Thirst\_AUC\_Predose\_Placebo = pre dose thirst ratings (AUC) on the VAS in the placebo condition
96. PreDose\_Arousal\_Insulin = pre dose arousal ratings (AUC) on the VAS in the insulin condition
97. PreDose\_Arousal\_Placebo = pre dose arousal ratings (AUC) on the VAS in the placebo condition
98. PreDose\_PhysicalEffects\_Insulin = pre dose physical effects ratings (AUC) on the VAS in the insulin condition
99. PreDose\_PhysicalEffects\_Placebo = pre dose physical effects ratings (AUC) on the VAS in the placebo condition
100. PreDose\_Appetite\_Insulin = pre dose appetite ratings (AUC) on the VAS in the insulin condition
101. PreDose\_Appetite\_Placebo = pre dose appetite ratings (AUC) on the VAS in the placebo condition
102. Pre\_Dose\_NegEffects\_insulin = pre dose negative effects ratings (AUC) on the VAS in the insulin condition
103. Pre\_Dose\_NegEffects\_Placebo = pre dose negative effects ratings (AUC) on the VAS in the placebo condition
104. PostDose\_Arousal\_Insulin = post dose arousal ratings (AUC) on the VAS in the insulin condition
105. PostDose\_Arousal\_Placebo = post dose arousal ratings (AUC) on the VAS in the placebo condition
106. PostDose\_PhysicalEffects\_Insulin = post dose physical effects ratings (AUC) on the VAS in the insulin condition
107. PostDose\_PhysicalEffects\_Placebo = post dose physical effects ratings (AUC) on the VAS in the placebo condition
108. PostDose\_Appetite\_Insulin = post dose appetite ratings (AUC) on the VAS in the insulin condition
109. PostDose\_Appetite\_Placebo = post dose appetite ratings (AUC) on the VAS in the placebo condition
110. Post\_Dose\_NegEffects\_insulin = post dose negative effects ratings (AUC) on the VAS in the insulin condition
111. Post\_Dose\_NegEffects\_Placebo = post dose negative effects ratings (AUC) on the VAS in the placebo condition
112. Thirst\_AUC\_Insulin\_PostDose = post dose thirst ratings (AUC) on the VAS in the insulin condition
113. Thirst\_AUC\_Placebo\_PostDose = post dose thirst ratings (AUC) on the VAS in the placebo condition