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Research report

High fat feeding promotes simultaneous decline in insulin sensitivity and cognitive performance in a delayed matching and non-matching to position task

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ABSTRACT

Obesity is the single greatest risk factor for the development of Type 2 diabetes mellitus (T2DM), with the prevalence of both dramatically increasing in recent years. These conditions are associated with medical complications such as hypertension, neuropathy and cardiovascular disease. Recent evidence also suggests a greater risk of developing dementia including Alzheimer's disease. The molecular mechanisms governing these changes remain obscure, although epidemiological evidence suggests that reduced insulin sensitivity (a characteristic of T2DM) is an independent risk factor for Alzheimer's disease. Here we examine the effects of diet-induced insulin resistance on cognitive ability in an animal model not predisposed to develop Alzheimer's pathology. Following 12 weeks on a high fat diet (45% of calories as crude fat) male Wistar rats were overweight and insulin resistant but not frankly diabetic. High fat fed animals were consistently poorer in all aspects of an operant based delayed matching to position task, yet were not impaired in spatial working memory as judged by the open field watermaze test. The cognitive deficit of the HF fed animals was most apparent when the task was switched from matching to non-matching to position, suggestive of an inability to change contingency. Performance in this task was negatively correlated with whole body insulin sensitivity but not weight gain. In conclusion this study has shown that insulin resistant animals exhibit impairments in an operant measure of behavioural flexibility which precede the development of diabetes.

1. Introduction

Type 2 diabetes (T2DM) accounts for more than 90% of all diabetes and this disorder has reached pandemic proportion in almost all regions of the world [41]. T2DM is often of insidious onset and is normally preceded by a period of raised fasting glucose or impaired glucose tolerance. These conditions can therefore be thought of as a pre-diabetic state and develop, at least in part, following a loss of sensitivity to the hormone insulin. Longitudinal studies reveal a higher risk of dementia or significant cognitive decline in diabetic and pre-diabetic populations [1,2,20,31,40 for reviews], while insulin resistance also increases the risk of mild cognitive impairment [40]. Insulin resistance is considered to be as powerful a cardiovascular and dementia risk factor as T2DM itself, and is strongly associated with lifestyle factors (including excess caloric intake and low levels of physical activity) that promote obesity [29,41]. Both hypertension and obesity correlate with poor cognitive performance in men [8], and obesity in middle age is a risk factor for developing dementia in later life [34]. In some studies only memory seems to be affected by diabetes-related factors [18] but in older populations, additional aspects of cognitive function may also be at risk [12].

In transgenic models of Alzheimer's disease (AD), such as the Tg2576 mouse (which expresses a mutant form of the human APP protein), high fat feeding induces insulin resistance and enhances the expression of the Aβ40 and Aβ42 fragments in brain extracts, peptides that aggregate into the characteristic amyloid plaques associated with AD [14]. In a combined Alzheimer and obesity mouse model (APP^+/-ob/ob) cognitive decline was exaggerated compared to the single APP^+ mouse, and was associated with increased insulin resistance but not increased brain amyloid β burden [32]. High fat feeding of a combined AD mouse model (a NSY mouse that spontaneously develops diabetes crossed with an Alzheimer model (APP23)) also worsened the learning impairment

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without affecting brain Aβ levels, suggesting that alterations in brain insulin signalling or some other consequences of diabetes may be better predictors of cognitive decline than Aβ levels [32].

The behavioural consequences of obesity and insulin resistance have been explored using the leptin receptor deficient rat (obese Zucker) and mouse (db/db) models of obesity induced type 2 diabetes. In the obese Zucker rat, performance on an operant task requiring alternation between response and non-response with a variable interval delay was impaired in conjunction with reduced GLUT4 translocation in the hippocampus [39]. However, learning deficits were less clear using a spatial learning task in the watermaze, with one study indicating an impairment in Zucker rats and db/db mice [17] but another detecting no deficit [5]. High fat feeding of rodents that are not genetically or pharmacologically predisposed to develop T2DM also has a detrimental effect on cognitive function, including performance on the radial arm maze, operant test of delayed alternation and a series of complex blind-alley maze tasks [11,38]. The molecular basis for these effects remains poorly characterised, but they suggest that factors associated with obesity can have detrimental effects on cognitive function independent of other vulnerability factors. Interestingly, performance in the radial arm maze and spatial-non-matching-to-sample improved following acute administration of glucose to aged rats [36], providing further evidence that alterations in glucose metabolism and/or insulin sensitivity play a vital role in cognition. It is generally assumed that peripheral insulin resistance (pre-diabetes) coincides with the development of neuronal insulin resistance, although this remains to be conclusively demonstrated at the molecular level [13,30].

The primary objective of this study was to investigate further the possible effects of high fat feeding on insulin sensitivity and cognitive function measured using an operant task which relies on sweetened rewards, and a spatial task requiring escape from water as the motivator.

2. Materials and methods

2.1. Subjects

All experiments were performed using male Wistar rats (Harlan UK limited) with an initial body weight of 150–175 g. Each behavioural task used a separate cohort of animals. The animals were housed in cages of four under a 12 h:12 h light:dark pattern (holding room lights on at 06:00 h; off at 18:00 h) at an ambient temperature of 22 ± 1 °C and 50% humidity. Rats had *ad libitum* access to either standard rat chow or a high fat diet except where otherwise stated. Water was freely available throughout the study. Weight was monitored weekly for the duration of the study. All experimental procedures were sanctioned by the University of Dundee Ethical Review Process and were performed in accordance with UK Home Office regulations under the auspices of Project Licence PIL60/3766.

2.2. Diet

Rats were randomly assigned by cage after initial training to receive either standard rat chow (SC; RM1-SDS diets, UK; kcal composition: 7.4% crude fat, 17.5% crude protein, 75.1% carbohydrate) or a high fat diet (HF; high fat diet-SDS 824053-; kcal composition; 45% crude fat, 20% crude protein, 35% carbohydrate). The carbohydrate and fat composition of the SC diet was rice starch (45%, w/w), sucrose (4.5%, w/w) and soya oil (2.71%, w/w) whereas the HF diet was rice starch (28.3%, w/w), sucrose (10.5%, w/w), lard (17.9%, w/w) and soya oil (4.3%, w/w). The animals remained on this diet for the duration of each experiment (12 weeks). For the operant and watermaze experiments the number of animals in the HF group was approximately 50% greater than that of the SC group because it was anticipated that a proportion of the animals on the HF diet would remain insulin sensitive and some of the animals would be unable to achieve task criterion.

2.3. Biochemical analysis

Fasting glucose and insulin were measured in all behavioural cohorts. For animals tested in the operant chambers (DMTP/DNMTP and Progressive ratio) or open field, glucose measurements we taken 0, 3, 6, 9 and 12 weeks and for the watermaze study samples were taken at 0, 3, 5, 9 and 12 weeks after commencing the diet. All samples were taken following an overnight fast (minimum 16 h) at least 4 days prior to any behavioural testing.

At each time point the saphenous vein of the hind limb was punctured and a drop (approximately 5 μl) sampled immediately using an Accuread© hand held glucose monitor. In addition at 0, 6 (week 5 in the watermaze study) and 12 weeks blood samples (20 μl) were collected into lithium-heparin coated microvette tubes (Starstedt UK) for the measurement of plasma insulin. Plasma was isolated following centrifugation (1000 g for 20 min) and insulin measured by ELISA (Ultra-Sensitive Rat Insulin ELISA Kit; Crystal Chem Inc, USA) using 5 μl in duplicate and following manufacturers guidelines.

2.4. Apparatus

2.4.1. Operant chambers

Tests were performed in a bank of eight operant chambers (Med Associates, UK). Each box was equipped with two retractable levers on either side of a central food hopper. A central house light permitted illumination of the chamber. A pellet dispenser delivered 45 mg sucrose pellet (Research Diets, Inc.; UK) to the food hopper on depression of the lever. Each chamber was controlled by on-line connection to a computer programmed using MedPC IV software.

2.4.2. Watermaze

Behavioural testing was performed in a large watermaze (2-m diameter) half-filled with water (26 ± 1 °C) made opaque with black poster paint. A clear platform of 12 cm diameter was submerged approximately 1 cm beneath the water. Extra maze cues were located around the pool to aid navigation. Swimming behaviour was monitored by an overhead video camera with an on-line data acquisition system (Watermaze© University of Edinburgh 1994).

2.5. Operant task

2.5.1. Habituation and training

Animals were maintained on a restricted food regime (85% of the free feeding daily intake per cage) for 3 days before training commenced. During this period SC rats were given 21 g food per rat per day; those on the HF diet were given 18 g of food per rat per day. Rats were habituated to the chambers and sucrose pellets. Following habituation animals were trained under a continuous reinforcement (CRF) schedule with the house light off. During this period both levers were extended and response on either lever was rewarded with delivery of a pellet. Each session was terminated after 40 min or when 100 pellets had been delivered. Criterion was set at 80 pellets over 3 consecutive days. On reaching criterion the animals progressed on to the next phase of training. During each trial only one lever was presented and this alternated between trials. Depression of the lever resulted in delivery of a sucrose pellet and retraction of the lever. Following a brief (5 s) inter-trial interval the opposite lever was presented. Training continued until a criterion of 80 correct responses over 3 consecutive days was achieved. Animals were then trained on a simple delayed matching to position (DMTP) task.

2.5.2. DMTP training and testing

For the operant DMTP task 28 naïve animals were used initially ($n = 8$ SC; $n = 20$ HF). Each DMTP session lasted for 40 min during which the house light remained off. The task began with a sample phase during which one lever was extended. This was predetermined by a computer programme so that both levers were presented approximately the same number of times. On depression of the sample lever, the lever retracted and an inter-trial interval (5 s) began. The first nose poke of the central food hopper after the ITI initiated the choice phase during which both levers were extended and a correct response (i.e. the sample lever) was rewarded with a sucrose pellet. An incorrect response resulted in a 5 s "time out" period during which both levers were retracted, the house light illuminated and no reward was delivered. Once a criterion of >80% correct choices was achieved over 3 consecutive days time delays of 0, 2, 4, 8, 12, 18 or 24 s were introduced at random between sample and choice phases. Upon reaching asymptomatic performance rats were tested for 5 days to provide baseline measurements. Animals were re-tested during weeks 10 and 11 on their respective diets. After 5 days of re-testing on the DMTP task, the contingency was switched to a non-matching paradigm which was identical to the DMTP task apart from during the choice phase, where a response to the opposite lever from that shown during the sample phase was rewarded, whereas a response to the same lever resulted in a time out phase. Animals were tested for 5 days on the DNMTP task.

2.5.3. Progressive ratio training and testing

For the progressive ratio task 16 naïve animals were used ($n = 8$ SC; $n = 8$ HF). Animals were habituated and trained on CRF as described above (Section 2.5.1). Training then progressed initially to a fixed ratio (FR) 1 with a 5 s time out (FR1/TO 5 s). Only one lever was rewarded (right and left levers were counter balanced between groups) and pressing the lever resulted in delivery of a sugar pellet followed by a 5 s time out (TO) where the lever became inactive. Following 3 successful days achieving 80 pellets, the schedule was increased to FR3/TO-5 s where 3 active lever presses per reward and finally FR5/TO-5 s (5 active lever presses per reward). All trials lasted for a maximum of 1 h or when 100 pellets had been delivered. Having reached the required criterion, the animals were placed on a progressive ratio (PR) schedule. The schedule during these tests was calculated using the formula $5*(\mathrm{EXP}(R*0.2)) - 5$