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ARTICLE

Susceptibility to diet-induced obesity and glucose intolerance in the $APP_{SWE}/PSEN1_{A246E}$ mouse model of Alzheimer's disease is associated with increased brain levels of protein tyrosine phosphatase 1B (PTP1B) and retinol-binding protein 4 (RBP4), and basal phosphorylation of S6 ribosomal protein

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Abstract
Aims/hypothesis Obesity is a major risk factor for development of insulin resistance, a proximal cause of type 2 diabetes and is also associated with an increased relative risk of Alzheimer's disease. We therefore investigated the susceptibility of transgenic mice carrying human mutated transgenes for amyloid precursor protein (APP_{SWE}) and presenilin 1 ($PSEN1_{A246E}$) (*APP*/*PSEN1*), or $PSEN1_{A246E}$ alone, which are well-characterised animal models of Alzheimer's disease, to develop obesity, glucose intolerance and insulin resistance, and whether this was age- and/or diet-dependent.
Methods We analysed the effects of age and/or diet on body weight of wild-type, *PSEN*1 and *APP*/*PSEN1* mice. We also analysed the effects of diet on glucose homeostasis and insulin signalling in these mice.
Results While there were no body weight differences between 16–17- and 20–21-month-old *PSEN1* mice, *APP*/*PSEN1* mice and their wild-type controls on standard, low-fat, chow diet, the *APP*/*PSEN1* mice still exhibited impaired glucose homeostasis, as investigated by glucose tolerance tests. This was associated with increased brain protein tyrosine phosphatase 1B protein levels in *APP*/*PSEN1* mice. Interestingly, short-term high-fat diet (HFD) feeding of wild-type, *PSEN1* and *APP*/*PSEN1* mice for a period of 8 weeks led to higher body weight gain in *APP*/*PSEN1* than in *PSEN1* mice and wild-type controls. In addition, HFD-feeding caused fasting hyperglycaemia and worsening of glucose maintenance in *PSEN1* mice, the former being further exacerbated in *APP*/*PSEN1* mice. The mechanism(s) behind this glucose intolerance in *PSEN1* and *APP*/*PSEN1* mice appeared to involve increased levels of brain retinol-binding protein 4 and basal phosphorylation of S6 ribosomal protein, and decreased insulin-stimulated phosphorylation of Akt/protein kinase B and extracellular signal-regulated kinase 1/2 in the brain.
Conclusions/interpretation Our results indicate that Alzheimer's disease increases susceptibility to body weight gain induced by HFD, and to the associated glucose intolerance and insulin resistance.

Keywords Diet-induced obesity · Insulin resistance · Glucose intolerance · Type 2 diabetes · Alzheimer's disease · Human mutations · Transgenes

Abbreviations

APP	Amyloid precursor protein
APP/*PSEN1*	Mice carrying human mutated transgenes for amyloid precursor protein (APP_{SWE}) and presenilin 1 ($PSEN1_{A246E}$)
ERK	Extracellular signal-regulated kinase
GTT	Glucose tolerance test
HFD	High-fat diet
PKB	Akt/protein kinase B
PSEN1	Presenilin 1
PTP1B	Protein tyrosine phosphatase 1B
RBP4	Retinol-binding protein 4

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Introduction

Alzheimer's disease, the most common type of progressive dementia in the elderly [1], is characterised by neurofibrillary tangles and deposits of β-amyloid. Three genes have been identified in familial early-onset Alzheimer's disease: (1) amyloid-β protein precursor (*AβPP*, also known as *APP*); (2) presenilin 1 (*PSEN1*); and (3) *PSEN2* [2]. The generation of transgenic mice with β-amyloid plaques has improved our understanding of pathophysiological processes associated with Alzheimer's disease [3, 4]. Thus, transgenic mice carrying human mutated transgenes for *APP*, *PSEN1* or *PSEN2*/*APP*+*PSEN1* mutated transgenes are a well-characterised animal model of Alzheimer's disease.

Increasing evidence suggests that insulin resistance, i.e. the inability of tissues to respond to the circulating effects of insulin, is a risk factor associated with pathogenesis of Alzheimer's disease [5]. Therefore, insulin-sensitising drugs such as the peroxisome proliferator-activated receptor γ agonist, rosiglitazone, have been found to improve cognitive function in transgenic *APP* mouse models of Alzheimer's disease [6]. Studies using analogues for the gut hormone glucagon-like peptide-1 (GLP-1) such as liraglutide, which facilitate insulin release, have shown that even short-term treatment can improve learning and memory, in addition to improving glucose homeostasis in mice with diet-induced obesity [7]. Moreover, an increase of GLP-1 signalling in the brain has also been suggested as a promising strategy to ameliorate the degenerative processes observed in Alzheimer's disease [8], while studies crossing *APP* mice to mouse models with alterations in brain insulin-signalling have revealed an important role for insulin signalling and glucose homeostasis in cognitive behaviour in these mice [9–11].

Currently, several potential mechanisms have been suggested that connect insulin resistance to Alzheimer's disease. One is that insulin crosses the blood–brain barrier from the periphery to the central nervous system and competes with β-amyloid for insulin degrading enzyme in the brain [12]. Peripheral hyperinsulinaemia may also inhibit brain insulin production, which, in turn, results in impaired amyloid clearance and a higher risk of Alzheimer's disease [13, 14]. Other mechanisms have also been suggested, such as advanced glycation end-products, which can be identified immunohistochemically in senile plaques and neurofibrillary tangles, the pathological hallmarks of Alzheimer's disease [15]. In addition, associations between adipokine levels and Alzheimer's disease have also been made [5]. Thus, obesity and the associated hyperinsulinaemia and hyperleptinaemia have been found to play an important role in early-onset learning deficits in *APP* mouse model of Alzheimer's disease, as crossing these mice to genetically obese *ob/ob* mice results in profound learning and memory deficits in mice as young as 8 weeks of age [16].

In our studies, we asked whether the presence of Alzheimer's disease genes was a risk factor for development of diet-induced obesity and glucose intolerance. Thus, we used the well-characterised double mutant mice carrying human mutated transgenes for amyloid precursor protein (APP_{SWE}) and presenilin 1 ($PSEN1_{A246E}$) (*APP*/*PSEN1*) mice, as these mice develop numerous amyloid deposits much earlier (9 months onwards) than age-matched mice expressing APP_{SWE} (positive at 18 months), $PSEN_{A246E}$ or wild-type mice [1]. In addition, behavioural and physiological deficits in *APP*/*PSEN1* mice are obvious, starting at around 6 months of age, prior to the presence of amyloid deposits, [3, 17]. We set out to investigate if the presence of Alzheimer's disease genes increases the risk of age- and diet-associated obesity and associated glucose intolerance and insulin resistance, by comparing aged *APP*/*PSEN1*, *PSEN1* and wild-type mice on standard rodent chow or when challenged with a high-fat diet (HFD).

Methods

Transgenic animals All animal studies were performed under a project licence approved by the Home Office under the Animals (Scientific Procedures) Act 1986. Mice were maintained on a 12 h light/dark cycle in a temperature-controlled barrier facility, with free access to water and food. Transgenic mice were purchased (Jackson Laboratories, Bar Harbor, ME, USA) and breeding colonies established at the University of Aberdeen, UK, as described previously [17].

For body weight analysis studies on chow diet, we used one cohort of male mice, which we weighed longitudinally (*APP*/*PSEN1* n=11, wild-type n=8 and *PSEN1* n=3). For the effects of chow vs HFD feeding on body weight gain and glucose tolerance, we used another cohort of male mice, which we examined pre- (i.e. while on chow) and post-HFD feeding (*APP*/PSEN n=18, *PSEN1* n=9 and wild-type n=8).

Metabolic measurements Tail blood glucose was determined using a Accu-Check glucometer (Roche Diagnotics, Mannheim, Germany). Glucose tolerance tests (GTT) were performed as described previously [18–21]. Briefly, mice were fasted overnight (16 h) and experiments started at 09:00 hours the following day. Mice were separated into individual cages, and their body weight and fasting blood glucose determined, followed by i.p. injection of a bolus of glucose (2 g/kg body weight). Blood glucose levels were

determined immediately before and at 15, 30, 60 and 120 min post-injection. For HFD studies, mice were placed on diet containing 55% kcal from fat, primarily hydrogenated vegetable shortening, TD93075 (Harlan Teklad, Madison, WI, USA) for a period of 8 weeks.

Biochemical analysis Whole-cell lysates were prepared by extraction in RIPA buffer (10 mmol/l TRIS-HCl, pH 7.4; 150 mmol/l NaCl; 0.1% [wt/vol.] SDS; 1% [vol./vol.] Triton X-100; 1% [wt/vol.] sodium deoxycholate; 5 mmol/l EDTA; 1 mmol/l NaF; 1 mmol/l sodium orthovanadate; protease inhibitors) at 4°C, followed by clarification at 14,000 *g*, as described previously [21]. For western blots, proteins were resolved by SDS-PAGE using pre-cast 18-well 10% gels (BioRad Laboratories, Hemel Hempstead, UK) and transferred to nitrocellulose membranes. Immunoblots were performed with rabbit polyclonal antibodies against phospho-AKT, phospho-extracellular signal-regulated kinase (ERK)1/ERK2, phospho-S6, mouse monoclonal ERK2 (all from Cell Signaling/New England Biolabs, Hitchin UK), protein tyrosine phosphatase 1B (PTP1B) (Upstate/Millipore, Watford, UK) and retinol-binding protein 4 (RBP4, A0040; Dako, Ely UK), following the manufacturer's directions. Proteins were visualised using enhanced chemiluminescence and quantified using a Fusion CCD imaging system and Bio1D software (Peqlab, Sarisbury Green, UK).

Statistical analysis Results are expressed as mean ± SEM. Comparisons between groups were made by ANOVA (one- or two-way, with repeated measures, where appropriate), with values of $p<0.05$ considered statistically significant.

Results

APP/PSEN1 mice are more susceptible to diet-induced body weight gain To investigate whether transgenic Alzheimer's disease mice are more susceptible to body weight gain with age, we analysed longitudinally ageing (16–17 and 20–21 months old) *APP*/*PSEN1* ($n=11$), wild-type ($n=8$) and *PSEN1* ($n=3$) mice for total body weight on a standard rodent, chow diet (Fig. 1). No differences in body weight were revealed between any of the genotypes; there was no indication of age-dependent changes (two-way repeated-measures ANOVA; $F<1$; $p>0.05$).

To investigate whether HFD feeding differentially affects body weight gain in *APP*/*PSEN1*, *PSEN1* and age-matched wild-type control littermate mice, we placed another cohort of 13-month-old *APP*/*PSEN1* ($n=18$), *PSEN1* ($n=9$) and wild-type ($n=7$) mice on HFD for a period of 8 weeks and recorded body weight gain during this time. There were no

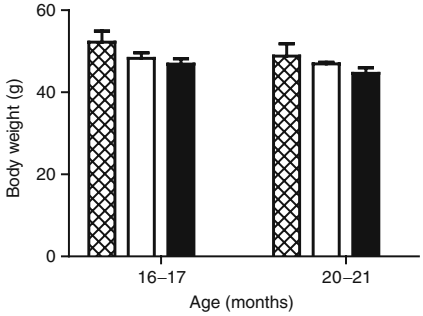

Fig. 1 Body weight of 16–17- and 20–21-month-old mice followed up longitudinally on standard chow diet. Wild-type, chequered bars, $n=8$; *PSEN1*, white bars, $n=3$; *APP*/*PSEN1*, black bars, $n=11$. No significant differences were observed ($p>0.05$ for all; $F<1$ for all)

statistical differences in total body weights between the three mouse groups before and after the HFD (Fig. 2a). However, both Alzheimer's disease genotypes gained weight significantly (*PSEN1* $p=0.03$; *APP*/*PSEN1* $p<0.001$), while weight gain in wild-type mice did not reach significance ($p=0.054$). The stronger significance obtained for the *APP* expressing mouse line was a first indication that this line was overall more prone to gain weight, as confirmed in Fig. 2b (gain of 13.1±1.1 g in *APP*/*PSEN1* mice vs 8.7±2.0 g in *PSEN1* mice and 8.2±3.7 g in wild-type mice, respectively), indicating that these mice are more susceptible to diet-induced body weight gain. In addition, overnight fasting resulted in greater body weight loss in wild-type mice (4.0±0.4 g) than in *PSEN1* (2.8±0.4 g) or *APP*/*PSEN1* mice (2.9±0.2 g), with significant differences observed between wild-type and *APP*/*PSEN1* mice (one-way ANOVA, $F=4.11$, $p=0.02$) (Fig. 2c).

Mouse models of Alzheimer's disease are susceptible to diet-induced hyperglycaemia, while presence of APP genotype leads to glucose intolerance To evaluate whether *PSEN1* and *APP*/*PSEN1* mice have impaired glucose homeostasis in comparison to their control wild-type mice, we first analysed their glucose homeostasis on a standard, chow diet. To investigate the effect of Alzheimer's disease-like phenotype on fasting hyperglycaemia, we withdrew food for a period of 16 h and measured fasting glucose levels. There were no differences between the groups on normal, low-fat standard chow diet (Fig. 3a). In contrast, short-term HFD-feeding significantly increased blood glucose levels in *APP*/*PSEN1* ($p<0.001$) and *PSEN1* ($p<0.05$) mice, with no significant effect on wild-type fasting hyperglycaemia ($p>0.05$) (Fig. 3a). Interestingly, blood glucose levels in overnight-fasted mice on HFD were significantly higher in *APP*/*PSEN1* mice than in *PSEN1* and wild-type controls, while *PSEN1* mice had significantly higher fasting glucose levels than wild-type mice, thereby exhibiting an intermediate phenotype (two-way repeated-measures ANOVA, interaction $F=4.82$; $p=0.01$) (Fig. 3a).

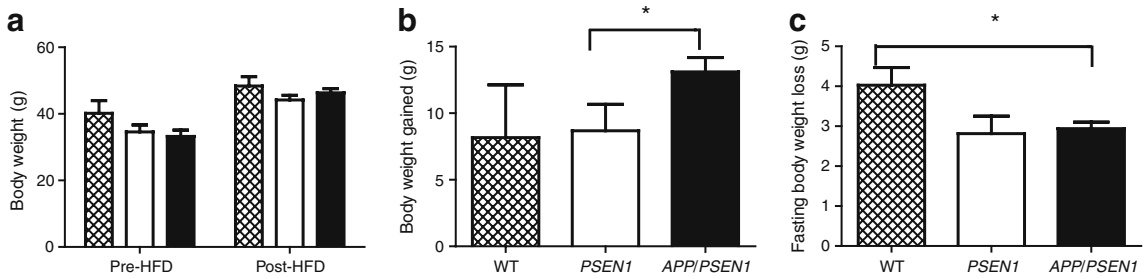

Fig. 2 **a** Body weight on standard chow and post HFD feeding. Wild-type (WT) controls, chequered bars, $n=7$; *PSEN1*, white bars, $n=9$; *APP/PSEN1*, black bars, $n=18$. There were no statistical differences in total body weight between the three mouse groups before and after HFD. However, while both Alzheimer's disease genotypes gained weight significantly (*PSEN1* $p=0.03$; *APP/PSEN1* $p<0.001$), wild-type mice weight gain did not reach significance ($p=0.054$). **b** Body weight gain in mouse groups described in **a** after 8 weeks of HFD feeding indicated a significant genotype difference; $p<0.05$ for *PSEN1* vs *APP/PSEN1* by one-way ANOVA. **c** Fasting body weight loss in mouse groups described in **a** after 8 weeks of HFD feeding indicated a significant genotype difference; $p<0.05$ for wild-type vs *APP/PSEN1* by one-way ANOVA

Since measuring circulating blood glucose levels is not the most comprehensive way to determine subtle differences in glucose homeostasis, we also performed GTTs in the mouse groups. On chow diet, no differences in 5 h (data not shown) or 16 h fasted circulating blood glucose levels (Fig. 3a) were observed, whereas GTTs revealed that *APP/PSEN1* mice were more glucose-intolerant than their wild-type controls, as evidenced by their slower rate of glucose clearance at 30 and 60 min post-GTT, and more intolerant than *PSEN1* mice at 60 min (two-way repeated-measures ANOVA, interaction $F=2.60$, $p=0.01$) (Fig. 3b). In addition, *PSEN1* mice exhibited an intermediate phenotype with worsening of glucose clearance significantly different from that in wild-type controls at 30 min post-GTT (Fig. 3b). Even though HFD-feeding induced glucose intolerance in all groups of mice, *APP/PSEN1* mice had much less efficient glucose clearance than their wild-type controls (Fig. 3c), with significance reached for glucose clearance after 30 min (two-way repeated-measures ANOVA). Again, *PSEN1* mice exhibited an intermediate phenotype, suggesting that presence of *APP* genotype is necessary for diet-induced glucose intolerance to be detectable. Analysis of the AUC (Fig. 3d) during a GTT further confirmed that *APP/PSEN1* mice are more glucose-intolerant than their wild-type control littermates with short-term HFD-feeding (two-way repeated-measures ANOVA, interaction $F=4.07$, $p=0.02$) (Fig. 3d).

Increased brain PTP1B and RBP4 protein levels and nutrient-driven basal S6 phosphorylation in mouse models of Alzheimer's disease are associated with glucose intolerance in these mice To investigate some of the potential mechanisms behind the increased glucose intolerance in *PSEN1* and *APP/PSEN1* mice, we determined insulin signalling

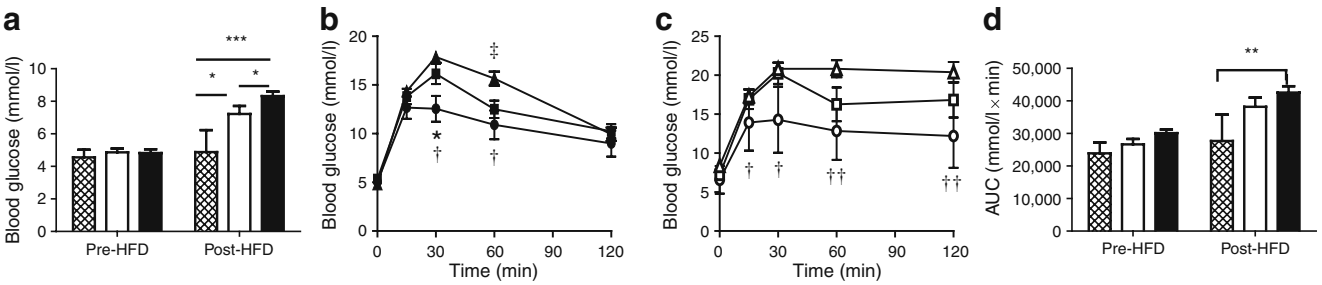

Fig. 3 **a** Overnight fasted blood glucose levels on chow and HFD. Wild-type controls, chequered bars, $n=8$; *PSEN1*, white bars, $n=9$; *APP/PSEN1*, black bars, $n=18$. While genotypes did not differ under chow conditions, HFD-feeding increased fasting blood glucose levels significantly in *PSEN1* and *APP/PSEN1* group only. However, this rise was more pronounced in the *APP/PSEN1* group; $^{*}p<0.05$ and $^{***}p<0.001$. In addition, *PSEN1* mice had higher fasting glucose levels than wild-type controls ($p<0.05$ by two-way ANOVA). *APP/PSEN1* mice had dramatically higher glucose levels than wild-type and *PSEN1* mice; $p<0.001$ and $p<0.05$, respectively, by two-way ANOVA. GTTs on chow (**b**) and (**c**) HFD. Wild-type controls, circles, $n=8$; *APP/PSEN1*, triangles, $n=18$; *PSEN1*, squares, $n=9$. On chow diet, *APP/PSEN1* mice were more glucose-intolerant than (1) wild-type controls, as evidenced by their slower rate of glucose clearance at 30 and 60 min post-GTT, and (2) *PSEN1* mice, as evidenced by their slower rate of clearance at 60 min post-GTT; $p=0.01$ by two-way repeated-measures ANOVA; interaction $F=2.61$. Significant differences between genotypes were as follows: $^{*}p<0.05$ for wild-type vs *PSEN1*; $^{\dagger}p<0.05$ and $^{\dagger\dagger}p<0.01$ for wild-type vs *APP/PSEN1*; $^{\ddagger}p<0.05$ for *PSEN1* vs *APP/PSEN1*. **d** Area under curve for GTTs pre- and post-HFD feeding (8 weeks) in mice. *APP/PSEN1* mice were more glucose-intolerant than wild-type controls after HFD-feeding; $^{**}p<0.01$ by two-way repeated-measures ANOVA

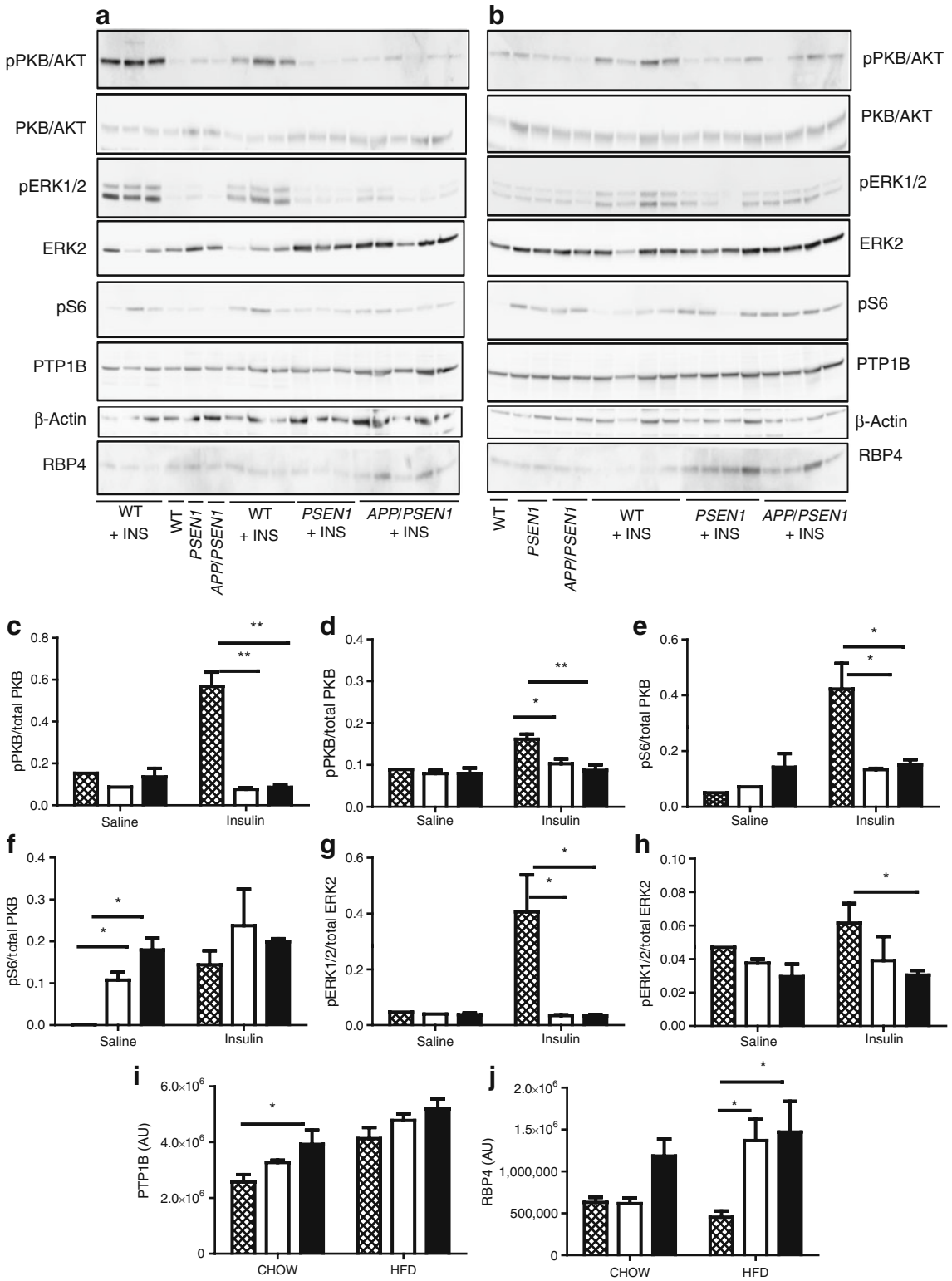

Fig. 4 Whole-cell lysates from brain (protein loading at 30 μg/lane) of wild-type (WT), *PSEN1* and *APP*/*PSEN1* mice fed (**a**) chow or (**b**) HFD, fasted overnight and injected with saline or insulin (10 min i.p., 10 mU/g) as indicated (insulin, +INS). Immunoblots were probed with pAkt/PKB (S473 site), total Akt/PKB, pERK1/2, total ERK2 (mouse monoclonal), pS6, PTP1B and RBP4 antibodies and visualised by enhanced chemiluminescence. Quantification of western blots, as indicated in Methods, from whole-cell lysates for (**c**) chow-fed and (**d**) HFD-fed brain pPKB/Akt (S473) normalised to total PKB/Akt. Quantification of blots for (**e**) chow-fed and (**f**) HFD-fed pS6 normalised to total PKB/Akt, and for (**g**) chow-fed and (**h**) HFD-fed pERK1/2 normalised to total ERK2. Quantification of blots for (**i**) PTP1B and (**j**) RBP4 protein levels. All statistics were analysed using two-way ANOVA; *$p<0.05$ and **$p<0.01$. **c**–**j** Chequered bars, wild-type; white bars, *PSEN1*; black bars, *APP*/*PSEN1*

components in mice fed chow (Fig. 4a) and HFD (Fig. 4b). Mice were fasted overnight and the following morning injected with a bolus of saline or insulin (10 mU/g body weight) for 10 min, followed by cervical dislocation and tissue extraction. Brains were homogenised in RIPA buffer as previously described [21], and brain insulin-signalling pathway analysed using phospho-specific antibodies.

Total protein levels of brain PTP1B, which is the major insulin receptor phosphatase and negative regulator of insulin and leptin signalling, were increased by Alzheimer's disease genotype on chow diet (Fig. 4a, i), while HFD-feeding increased brain PTP1B levels in all groups of mice (Fig. 4b, i), consistent with previous findings [22].

Serum RBP4 is an adipokine found to be elevated in insulin-resistant humans and in many mouse models of obesity and insulin resistance, including HFD [23], although not all studies show this effect [24]. According to many studies, the level of elevation correlates highly with the degree of insulin resistance in mice and in humans, and serum RBP4 levels were highly predictive of metabolic syndrome risk in a large population-based study [25]. We analysed the levels of brain RBP4 in our mice on chow and HFD. Our data suggest that chow-fed *APP*/*PSEN1* mice tend to have increased levels of brain RBP4 in comparison to wild-type and *PSEN1* mice (Fig. 4a, j), while HFD-feeding significantly increases brain RBP4 levels in *PSEN1* and *APP*/*PSEN1* mice, relative to wild-type control littermates (two-way ANOVA) (Fig. 4b, j).

We also examined nutrient- and insulin-regulated S6 ribosomal protein phosphorylation, finding that in mice on HFD, basal S6 phosphorylation was dramatically higher in brains from *PSEN1* and *APP*/*PSEN1* mice (Fig. 4b, f) and was as high as wild-type insulin-stimulated S6 phosphorylation (two-way ANOVA). This was not the case on standard low-fat chow diet (Fig. 4a, e).

Given that recent data have shown that crossing *APP* mice to *ob/ob* mice resulted in complete absence of phosphorylation of Akt/protein kinase B (PKB) at S473 site in muscle and liver, we also investigated phosphorylation status of this site in brains from our mice. While insulin was able to stimulate phosphorylation of Akt/PKB on S473 site in wild-type mice, which was somewhat diminished with short-term HFD feeding (Fig. 4b–d), insulin was not able to cause any phosphorylation of Akt/PKB in *APP*/*PSEN1* or *PSEN1* mice on chow diet (Fig. 4a, c), or on HFD (two-way ANOVA) (Fig. 4b, c). Phosphorylation of ERK1/2 was strikingly higher in insulin-stimulated brains from wild-type mice fed chow diet (Fig. 4a, g) and HFD (Fig. 4b, h) than in *PSEN1* and *APP*/*PSEN1* mice on the same diets.

Discussion

Obesity is a major risk factor for the development of several disorders such as type 2 diabetes, metabolic syndrome, cardiovascular disease, dementia and cancer. In addition, due to improvements in medicine, housing, economic factors and general well-being in the developed countries, people are living much longer. Ageing is also associated with the induction of most of these diseases, with associated costs to healthcare systems in the developed world also rapidly raising. Alzheimer's disease is the most common form of dementia, characterised by loss of recent memory as one of the first symptoms and a progressive decline in all cognitive skills later on in the disease [3, 26]. Considerable evidence suggests that insulin resistance and obesity may directly contribute to the pathogenesis of Alzheimer's disease [27–31]. However, not much emphasis has been put on the role of Alzheimer's disease in obesity and type 2 diabetes development, as characterised by insulin resistance. In this study, we set out to investigate, in a well-established *APP*/*PSEN1* and *PSEN1* mouse model, whether genotypes relevant to Alzheimer's disease make mice more susceptible to obesity development than their wild-type control littermates with increasing age and under different dietary conditions (low-fat standard chow diet vs HFD). We found that aged *PSEN1* and *APP*/*PSEN1* mice weighed exactly the same as their wild-type control mice, so ageing on its own did not seem to make Alzheimer's disease mice more susceptible to increased body weight gain in comparison to their controls. However, even a short-term dietary treatment with HFD, consisting of 55% fat, made *APP*/*PSEN1* mice significantly more susceptible to body weight gain than *PSEN1* littermates or wild-type controls. Thus, *APP* genotype is a risk factor for diet-induced body weight gain, with potential implications with regard to other secondary co-morbidities that may develop with an increase in body weight, e.g. type 2 diabetes. Indeed, our HFD study is in agreement with a similar study using sucrose-rich diet (10% sucrose) as a dietary supplement, which also led to an increase in body weight gain in *APP*/*PSEN1* mice [27]; however, that study only used non-supplemented diet vs supplemented diet in *APP*/*PSEN1* mice. No wild-type or transgene alone mice were included for a comparison. Our aim, however, was to determine whether the *APP* gene was the risk factor for increase in body weight gain or just diet alone.

Thus, we next set out to investigate whether an Alzheimer's disease-like phenotype would make mice glucose-intolerant and insulin-resistant, and whether this was controlled by dietary conditions. Interestingly, even though we observed no differences in body weight between *APP*/*PSEN1*, *PSEN1* and wild-type mice on chow diet, *APP*/*PSEN1* mice were significantly more glucose-

intolerant than *PSEN1* transgene mice and/or their wild-type controls. However, at this point, *APP*/*PSEN1* mice were still normoglycaemic, suggesting that we detected early changes in glucose maintenance. However, glucose intolerance induced by diet-induced obesity was strikingly evident in *APP*/*PSEN1* mice, as they also developed diet-induced fasting hyperglycaemia after 8 weeks of HFD feeding. This was accompanied by worsening in glucose maintenance as measured by GTTs. In addition, *PSEN1* transgene alone mice exhibited an intermediate phenotype, with fasting hyperglycaemia present under HFD feeding conditions. They were, however, significantly more glucose-tolerant than their *APP*/*PSEN1* control littermates. Thus, presence of *APP* genotype presents a risk factor for development of glucose intolerance under normal dietary conditions as well as under HFD-feeding.

To investigate the potential mechanism(s) associated with this glucose intolerance, we examined the levels of PTP1B, a well-known negative regulator of insulin and leptin receptor signalling [32] whose levels are elevated in obesity in humans [33, 34] and rodents, and under inflammatory conditions [22, 35, 36]. Mice lacking PTP1B globally or specifically in the brain [35, 37, 38] are lean, while those lacking muscle- or liver-PTP1B are protected against HFD-induced insulin resistance and glucose intolerance [18, 19]. Interestingly, we found brain PTP1B protein levels to be increased in *APP*/*PSEN1* mice on chow diet in comparison to wild-type controls, with *PSEN1* mice having an intermediate phenotype. As expected, HFD feeding increased PTP1B levels in the brain in all groups of mice, with no statistical differences between groups. However, brain RBP4 protein levels tended to be higher in *APP*/*PSEN1* mice on chow diet and significantly higher in *PSEN1* and *APP*/*PSEN1* mice on HFD, suggesting that increased RBP4 levels may be another mechanism by which Alzheimer's disease genotype predisposes to diet-induced glucose intolerance. RBP4 is an adipokine that was recently found to be elevated in insulin-resistant states as well as in obesity in mice [23, 39, 40] and humans [41–44], although not all studies have reported this [24, 45]. These data suggest that there are early markers of insulin resistance already present with the *APP* genotype, making these mice more susceptible to the effects of HFD feeding and associated insulin resistance. They also raise an interesting therapeutic possibility that reduction PTP1B and/or RBP4 levels by pharmacological means [39] may in turn protect against body weight gain and the associated glucose intolerance in Alzheimer's disease mouse models. This could be an interesting avenue for further studies.

In addition, we investigated brain insulin-signalling events in all groups of mice. Mice were fasted overnight and injected with a bolus of saline or insulin. Basal and insulin-stimulated phosphorylation events were then examined under chow and HFD feeding conditions. Since HFD is known to promote insulin resistance through nutrient overload-associated increase in S6K1 activity, we used phosphorylation of S6 as a read-out of S6K1 activity [46]. We found that under HFD feeding conditions, basal S6 phosphorylation was significantly higher in brains from *PSEN1* and *APP*/*PSEN1* mice and was as high as the insulin-stimulated S6 phosphorylation observed in wild-type mice, consistent with the insulin resistance observed in Alzheimer's disease mice. Since a decrease in insulin-stimulated Akt/PKB phosphorylation was observed in other mouse models of Alzheimer's disease in specific regions of brain or peripheral tissues [16, 28], we examined Akt/PKB phosphorylation from whole-brain lysates, finding, as expected from their impaired glucose tolerance, that insulin-stimulated Akt/PKB phosphorylation was decreased in *PSEN1* and *APP*/*PSEN1* mice under chow as well as HFD feeding conditions. In addition, insulin-stimulated phosphorylation of ERK1 and -2 was downregulated in *PSEN1* and *APP*/*PSEN1* mice in comparison to wild-type controls, both on chow and HFD. Thus it appears that in our Alzheimer's disease mice, insulin-stimulated Akt/PKB phosphorylation was inhibited, while nutrient-driven S6K signalling became augmented.

In conclusion, we report here that transgenic Alzheimer's disease mice are susceptible to diet-induced body weight gain, and the associated glucose intolerance and fasting hyperglycaemia, which are associated with increased brain PTP1B and RBP4 levels, and nutrient-driven basal S6 phosphorylation, as well as with decreased insulin-stimulated Akt/PKB and ERK1/2 phosphorylation. Our data suggest that Alzheimer's disease is a risk factor for diet-induced body weight gain and associated glucose intolerance, and that Alzheimer's disease patients should be advised to avoid foods high in fat, in order to prevent development of obesity-associated co-morbidities.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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