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Irs2 deficiency alters hippocampus-associated behaviors during young adulthood

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Abstract

Type 2 diabetes mellitus (T2DM), characterized by hyperglycemia and insulin resistance, has been recognized as a risk factor for cognitive impairment and dementia, including Alzheimer's disease (AD). Insulin receptor substrate2 (IRS2) is a major component of the insulin/insulin-like growth factor-1 signaling pathway. *Irs2* deletion leads to life-threatening T2DM, promoting premature death in male mice regardless of their genetic background. Here, we showed for the first time that young adult male mice lacking Irs2 on a C57BL/6J genetic background ($Irs2^{-/-}$/6J) survived in different experimental environments and displayed hippocampus-associated behavioral alterations. Young adult male $Irs2^{-/-}$6J mice also exhibit aberrant alterations in energy and nutrient sensors, such as AMP-activated protein kinase (AMPK) and glucose transporter3 (GLUT3), and reduced core body temperature accompanied by abnormal change in the temperature sensor in the brain. These results suggest that Irs2 deficiency-induced impairments of brain energy metabolism and thermoregulation contribute to hippocampus-associated behavioral changes in young adult male mice.

Keywords

Irs2; Type2 diabetes; Insulin; Behavior; Hippocampus; AMPK; GLUT3; TRPV4

1. Introduction

Of the main four IRS family proteins, Irs2 is ubiquitously expressed in most tissues as well as Irs1 [1]. Male mice lacking Irs2 on a mixed C57BL/6 × 129sv genetic background ($Irs2^{-/-}$/129 mice) die around 17 weeks of age due to severe type 2 diabetes mellitus (T2DM) and insulin resistance accompanied by dehydration and hyperosmolar

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Declaration of competing interest

The authors declare no conflicts of interest.

Appendix A. Supplementary data

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coma, whereas female *Irs2*$^{-/-}$/129 mice with mild diabetes survive for longer than 6 months [2,3]. Then, mice with the deletion of *Irs2* have been maintained on a C57BL/6J genetic background (*Irs2*$^{-/-}$/6J mice) after more than six backcrosses [4]. Compared to the *Irs2*$^{-/-}$/129 mice, the mice with the *Irs2* deletion on a C57BL/6J genetic background (Irs2$^{-/-}$/6J) die prematurely, between 8 and 12 weeks, due to extreme diabetes symptoms [4]. Conversely, the *Irs2*$^{-/-}$/6J females with mild T2DM and *Irs2*$^{-/-}$/129 females survive for greater than 4 months (D.T and A.T., unpublished data).

T2DM is associated with an increased risk factor of cognitive impairment, dementia, including Alzheimer's disease (AD) [5]. Although some studies have suggested that brain insulin resistance and dementia share features, other studies have shown that the two diseases are unrelated [6]. Thus, the detailed mechanisms underlying the link between the two diseases remain largely unknown. In addition, brain insulin resistance is not yet defined. The increased phosphorylation of IRS1 at well-known serine (Ser) residues, such as human(h)Ser312/mouse(m)Ser307 and hSer616/mSer612 that are associated with insulin sensitivity, has been found in the brains of patients or mice with AD and in the middle-aged high-fat diet/diet-induced obesity (DIO)-induced physiological model mice for T2DM (middle-aged DIO mice) showing amyloid-β (Aβ)-unrelated cognitive decline; however, it occurs regardless of the presence or absence of hyperglycemia and insulin resistance [7–9].

Although 12-week-old female *Irs2*$^{-/-}$/BL 6 mice exhibit impaired synaptic activation of N-methyl-D-aspartate receptor but normal basal synaptic transmission [10], 7-week-old male Irs2−/−/6J mice (from pubertal stage to early adolescence)display dopamine-associated behavioral changes. However, the physiological and behavioral phenotypes during young adulthood remain unknown because those male mice die between 8 and 12 weeks [4,11]. However, the male Irs2−/−/6J mice created in our current experimental environment live longer than those created in former or other experimental environments in different institutes [4,11]. In other words, male *Irs2*$^{-/-}$/6J mice survive more than 10 weeks but fewer than approximately 24 weeks, allowing us to study their physiological and behavioral alterations.

In the present study, we examined the energy metabolism and hippocampus-associated cognitive functions in 13- to 20-week-old (young adult) male *Irs2*$^{-/-}$/6J mice. The deletion of *Irs2* on a C57BL/6J background was found to exert different effects on food intake and circulating leptin level that are associated with energy homeostasis compared to Irs2 deletion in the mixed C57BL/6 × 129sv background [2,3]. Conversely, impaired glucose metabolism was found to occur regardless of genetic backgrounds and experimental environments. We found that the deletion of *Irs2* led to hippocampus-associated behavioral alterations that were not observed during pre- and early adolescence in young adult male wild-type (WT) mice without the modification of hippocampal Irs1. Furthermore, Irs2 deficiency-induced behavioral deficits during young adulthood were accompanied by impaired brain energy metabolism with aberrant changes in energy and nutrient sensors and thermoregulatory dysfunction with abnormal alterations in temperature sensor in the brain.

2. Materials and methods

A full description of the materials and methods can be found in the Supplementary materials and methods in Supplementary information.

3. Results

3.1. Irs2 deficiency leads to T2DM in mice regardless of genetic background and experimental environment

Similar to the male *Irs2*$^{-/-}$/129mice, the male *Irs2*$^{-/-}$/6J mice weighed less than male WT mice until 6 weeks; however, there was no significant alteration in body weight between the *Irs2*$^{-/-}$/6J and WT mice on a C57BL/6J genetic background after 7 weeks (Fig. 1A). Meanwhile, persistent low body weight was observed in Irs2$^{-/-}$/129 mice [3]. Moreover, unlike the male *Irs2*$^{-/-}$/129 mice [3], the fed blood glucose levels of the male *Irs2*$^{-/-}$/6J mice were not significantly different from those in the WT mice between 4 and 8 weeks. By contrast, the *Irs2*$^{-/-}$/6J males developed hyperglycemia after 9 weeks (Fig. 1B). Consistent with these results, glucose intolerance in *Irs2*$^{-/-}$/6J males was detected using the intraperitoneal (i.p.) glucose tolerance test (GTT) after 13 weeks (Fig. 1C). Meanwhile, the *Irs2*$^{-/-}$/6J males displayed higher fasting insulin levels and insulin resistance during the insulin tolerance test (ITT) than the WT males. (Fig. 1D and E). Additionally, the deletion of *Irs2* in mice on a C57BL/6J background did not affect the circulating leptin levels and food intake (Fig. 1F and G). This observation is consistent with the above result indicating no difference in body weight between the *Irs2*$^{-/-}$/6J and WT mice after 7 weeks (Fig. 1A). These data indicate that, under our experimental environment, Irs2 deficiency on a C57BL/6J background causes T2DM regardless of genetic backgrounds and experimental conditions but has distinct effects on growth and energy homeostasis in male mice compared with those on a mixed C57BL/6 × 129sv background.

3.2. Young adult male Irs2$^{-/-}$/6J mice display hippocampus-associated behavioral changes

We examined the hippocampal-associated behaviors in young adult male *Irs2*$^{-/-}$/6J mice at 13–18 weeks. As with the *Irs2*$^{-/-}$/6J males at 7 weeks (during pre- and early adolescence), the 13- to 18-week-old (young adult) *Irs2*$^{-/-}$/6J males exhibited decreased spontaneous activity in the open field; however, there was no significant difference in spontaneous alternation in the Y-maze between the groups (Fig. 2 A and C). Interestingly, the *Irs2*$^{-/-}$/6J males exhibited an increase in anxiety-like behavior, which was undetected at 7 weeks, that occurred during young adulthood in the elevated plus maze test (Fig. 2 B). Furthermore, the water T-maze test results [8,9] revealed that the young adult male *Irs2*$^{-/-}$/6J mice displayed impairment of hippocampus-dependent spatial memory. Conversely, there was no significant difference between the WT and *Irs2* KO mice in passive avoidance memory and hippocampal neurogenesis, which were positively correlated with hippocampus-related memory functions (Fig. 2 D and E; Suppl. Fig. 1A and B). These results indicate that deletion of *Irs2* in male mice exerts a negative impact on hippocampus-related emotional responses and spatial memory during young adulthood.

3.3. Aberrant activation of metabolic energy sensor related to low energy conditions in the hippocampus of young adult male Irs2$^{-/-}$/6J mice

Next, we investigated the alterations in insulin signal transduction in the hippocampus of young adult male *Irs2*$^{-/-}$/6J mice showing hippocampus-related behavioral changes (Fig. 2B and E). We previously demonstrated that memory impairment in middle-aged DIO mice was accompanied by increased phosphorylation of hippocampal IRS1 at specific Ser residues [8,9]. Regardless of the presence or absence of insulin, the phosphorylation levels of hippocampal IRS1 at mSer307 and mSer612 sites increased in middle-aged DIO mice were comparable between WT and *Irs2*$^{-/-}$/6J mice. By contrast, the phosphorylation of IRS1 at mSer632/635 or mSer1097 was elevated in middle-aged DIO or aged mice but reduced in the hippocampus of the KO males (Fig. 3A) [8,9].

Consistent with previous studies [8,9], there were no significant differences in the activation of canonical downstream kinases, such as p44/42 MAPK (Erk1/2), Akt/protein kinase B, glycogen synthase kinase 3 beta (GSK3β), mammalian target of rapamycin (mTOR), and ribosomal protein S6 kinase (S6K) between the WT and KO males regardless of the presence or absence of insulin. Meanwhile, the insulin-stimulated activation of Akt was substantially reduced in the liver and muscles of KO mice compared with those in the WT mice (Fig. 3B; Suppl. Fig. 2A and B).

Conversely, insulin administration had no impact on AMPK activation induced under low-energy conditions, such as fasting, in the WT mice. However, the deletion of *Irs2* induced the aberrant activation of AMPK in the hippocampus during insulin stimulation. Meanwhile, the level of adenosine triphosphate (ATP) decreased significantly in the hippocampal tissue following its abnormal elevation in the fasted state, despite the subtle alterations in the production of mitochondrial proteins, such as increased COX III, decreased cytochrome *c*, and decreased pyruvate dehydrogenase (Fig. 3 B and C; Suppl. Fig. 3B). Together, these results indicate that the modifications of hippocampal IRS1 observed in the physiological T2DM mouse model showing hippocampus-dependent memory deficits are not involved in the hippocampus-associated behavioral impairments in young adult male *Irs2*$^{-/-}$/6J mice, where the impaired brain energy metabolism is accompanied by the aberrant alterations of AMPK and tissue ATP.

3.4. Inactivation of Irs2 in mice downregulates body temperature and its related factors in the brain during young adulthood

Previous studies showed that when AMPK is activated, the level lactate, a product of glucose metabolism generated via glycolysis is increased, or the level of glucose transporter1 (GLUT1) and GLUT3, crucial in glucose uptake in astrocytes or neurons, is decreased, or both, in the brains of patients and animals with AD or diabetes [12,13]. Consistently, decreased levels of lactate and increased levels of GLUT1 and GLUT3 were observed in the hippocampus of WT mice with insulin stimulation following fasting (Fig. 4A and B), whereas the activity of AMPK was unchanged regardless of insulin stimulation (Fig. 3B). Conversely, Irs2 deficiency failed to decrease the lactate levels and increase the GLUT3 levels during insulin stimulation, despite the unchanged level of GLUT1 and the plasma level of catecholamines, including adrenaline, noradrenaline, and dopamine, that influence

the level of lactate [14] (Fig. 4A and B; Suppl. Fig. 3A). These data indicate that the deletion of *Irs2* in mice induces aberrant changes in lactate and GLUT3 levels accompanied by the abnormal activation of AMPK in the hippocampus of young adult male mice during insulin stimulation, which reflects impaired brain energy metabolism.

Because of the correlation between energy metabolism and body temperature [15,16], the effect of Irs2 deficiency, which causes impaired neural energy status, on rectal temperature was examined. The core body temperatures of *Irs2*$^{-/-}$/6J males were modestly but significantly decreased compared with those in the WT males regardless of their feeding status (Fig. 4C). Transient receptor potential vanilloid 4 (TRPV4) is a member of the thermosensitive transient receptor potential (TRP) family. It is activated by physiological temperature (>27–34 °C) and 4alpha-phorbol 12,13-didecanoate (4alpha pdd) and is associated with regulating energy homeostasis and neural activity in the hippocampus [17,18]. The alteration of TRPV4 was investigated in the hippocampus of the mice. We found that insulin increased the level of TRPV4 in the hippocampus of the WT mice, whereas Irs2 deficiency significantly reduced the insulin-stimulated production of TRPV4. By contrast, the basal level of TRPV4 was comparable in the WT and *Irs2* KO mice without insulin (Fig. 4E). These results suggest that Irs2 is involved in regulating core body temperature; its inactivation in mice leads to temperature dysregulation and the downregulation of the insulin-induced production of hippocampal TRPV4.

4. Discussion

In this study, we have demonstrated, for the first time, that young adult male *Irs2*$^{-/-}$/6J mice under different environmental conditions display hippocampus-associated behavioral changes while exhibiting T2DM. Irs2 deficiency-induced behavioral changes arise independently of the modification of hippocampal IRS1, and are accompanied by aberrant alterations in energy and nutrient sensors of the hippocampus that reflect impaired brain energy metabolism. In parallel, thermoregulatory dysfunction is observed in young adult male *Irs2*$^{-/-}$/6J mice.

Genetic backgrounds and environmental factors, including diet, water, and air, could affect the phenotypes of genetically engineered mice [19,20]. In our experimental environment, *Irs2*$^{-/-}$/6J males live longer than the *Irs2*$^{-/-}$/129 males and the *Irs2*$^{-/-}$/6J males in different environmental conditions. As a result, we could investigate the metabolic and neurological phenotypes, including behaviors, in young adult male *Irs2*$^{-/-}$/6J mice. However, the *Irs2*$^{-/-}$/6J males finally die approximately 24 weeks as well as the *Irs2*$^{-/-}$/129 males die prematurely (D.T and A.T., unpublished data), suggesting that Irs2 deficiency can lead to life-threatening T2DM in male mice irrespective of genetic background and environmental conditions.

Although young adult male *Irs2*$^{-/-}$/6J mice display hippocampus-related memory deficits and anxiety-like behavior that is a trigger for depression without the phosphorylation of IRS1 at specific Ser sites that is observed in the hippocampus of middle-aged DIO mice [8,9,21], the monotonous activity of Akt and GSK3b in the hippocampus is commonly observed in both the *Irs2*$^{-/-}$/6J and DIO mice [8,9]. These data suggest that memory

impairment with T2DM occurs independently of the activation of canonical downstream kinases, such as Akt and GSK3b, regardless of causes of T2DM.

Growing evidence suggests that chronic impairment, i.e., the increase or decrease, of brain energy metabolism induced by impaired nutritional status, including T2DM, is implicated in the pathogenesis of cognitive dysfunction [9,22]. Consistently, cognitive dysfunction induced by Irs2 inactivation is accompanied by the impairment of brain energy metabolism with aberrant alterations in metabolic energy sensors, such as AMPK and GLUT3. Meanwhile, thermoregulatory disorder with insulin-induced dysregulation of TRPV4 occurs in the brain of young adult *Irs2*$^{-/-}$/6J males. Importantly, these alterations caused by Irs2 deficiency are undetected in middle-aged DIO mice [8,9,22] (D.T and A.T., unpublished data), suggesting distinct mechanisms by which the deletion of *Irs2* causes behavioral impairments during young adulthood compared to those in middle-aged DIO mice. Of note, thermoregulatory disorder observed in various neurological disorders, including AD, can be arisen from the preclinical stage of diseases [23]. In connection with this, the deletion of TRPV4 leads to behavioral impairments hinder neural activity in animals [24]. Therefore, our data support the possibility that, in *Irs2*$^{-/-}$/6J male mice, an aberrant decrease of TRPV4 protein in the hippocampus during insulin stimulation may be associated with the onset of behavioral alterations in young adulthood.

In summary, the present study demonstrates that Irs2 deficiency in mice leads to impaired energy homeostasis and thermoregulatory dysfunction in the brain, likely decreasing spatial memory and abnormal emotional responses in the hippocampus during young adulthood. However, whether the deletion of *Irs2* or T2DM induced by Irs2 deficiency causes these behavioral changes in male mice during young adulthood remains unclear. Because behavioral impairments occur in pre- and early adolescent *Irs2*$^{-/-}$/6J males who already have hyperglycemia [11], it is difficult to evaluate these two effects on behaviors separately. Alternatively, Irs2 deficiency improves behavioral impairments and delays premature death in the AD mouse model in the absence of hyperglycemia. This finding is consistent with previous studies showing that the beneficial effects of brain-specific *Irs2* deletion in *BIrs2*$^{-/-}$/6J mice were unaccompanied by hyperglycemia but not hyperinsulinemia on life span and spatial memory but not intrinsic excitability regardless of proportionally reduced brain size in both *Irs2*$^{-/-}$/6J and *BIrs2*$^{-/-}$/6J mice [4,25–29], whereas hyperglycemic AD male mice with Irs2 deficiency die before 8 months [26]. Given these results in mice, it is reasonable to assume that behavioral impairments in young adult *Irs2*$^{-/-}$/6J males are caused by Irs2 inactivation accompanied by T2DM. Nonetheless, our data do not exclude the possibility that other factors contribute to neurological alterations including the behavioral impairments in the context of Irs2 deficiency during young adulthood. Future studies using animal models with a stage-specific mutation in specific regions and cells combined with pharmacological treatment with diabetes medications and the use of human tissues, such as postmortem brains from patients with cognitive dysfunction with or without T2DM, will help understand the roles of Irs2 in hippocampus-associated cognitive functions, including spatial memory and emotional responses, and identify the molecular signatures in these disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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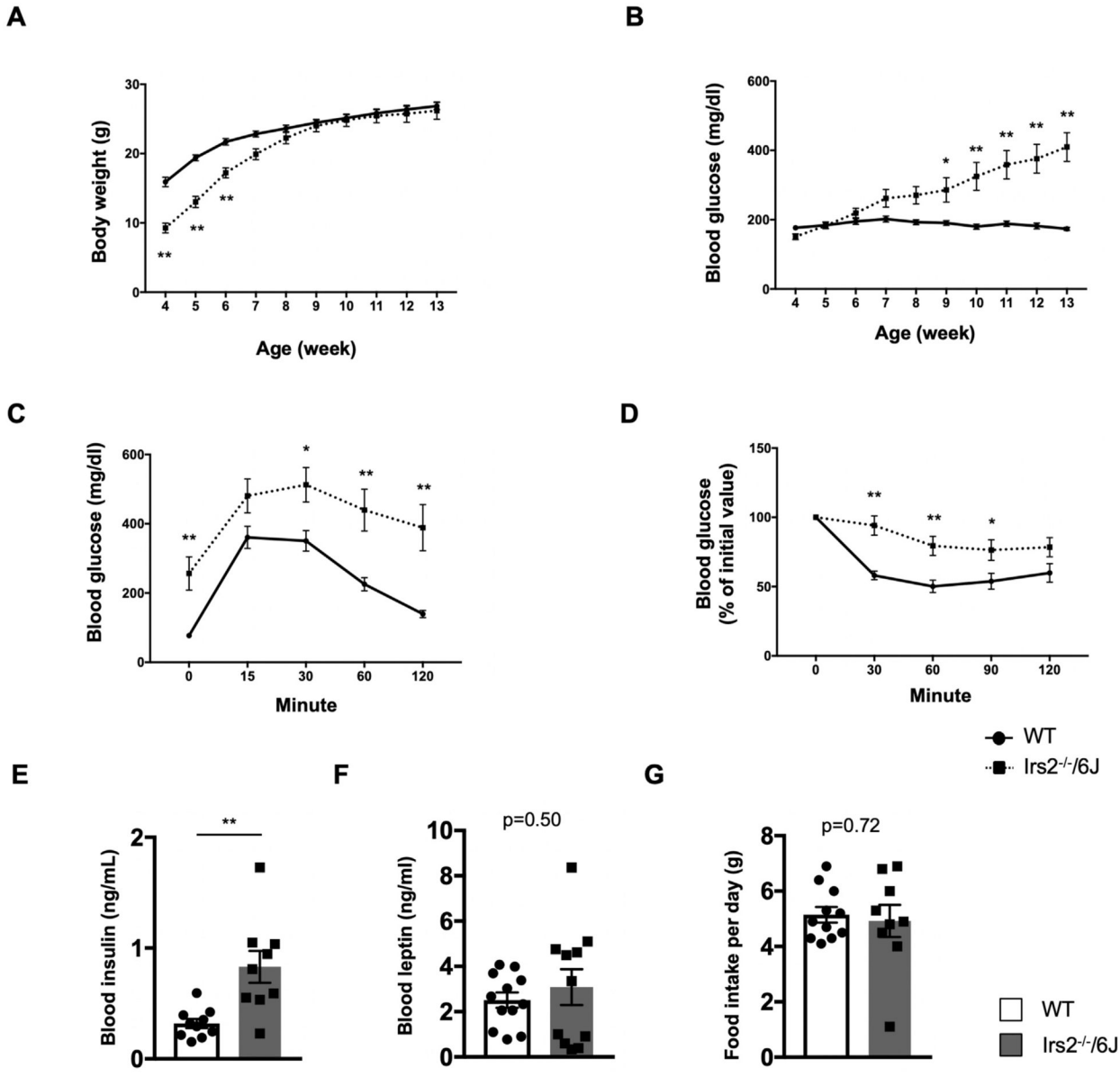

Fig. 1. Alterations in growth and energy homeostasis in male *Irs2*$^{-/-}$/6J mice.

(A) Body weight and (B) randomly fed blood glucose levels in the WT and *Irs2*$^{-/-}$/6J mice (n = 14 per group). (C) The GTT and (D) The ITT in both mice (13–18 weeks, n = 8–11 per group). (E) Fasting blood insulin levels, (F) fasting blood leptin levels, and (G) food intake in both mice (13–18 weeks, n = 9–12 per group). Results are presented as mean ± standard error of the mean (SEM), *$p < 0.05$; **$p < 0.01$.

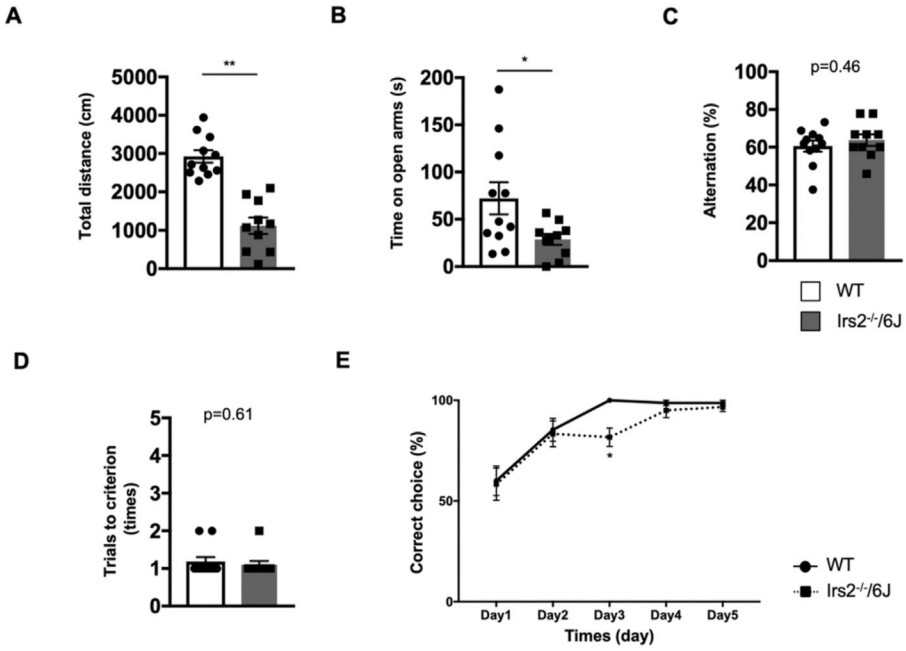

Fig. 2. Hippocampus-associated behavioral changes in young adult male *Irs2*$^{-/-}$/6J mice.

(A) Open field, (B) elevated plus maze, (C) Y maze, (D) passive avoidance testing, and (E) the water T maze test in the WT and *Irs2*$^{-/-}$/6J mice (13–18 weeks, n = 10–15 per group). Results are presented as mean ± standard error of the mean (SEM); *$p < 0.05$; **$p < 0.01$.

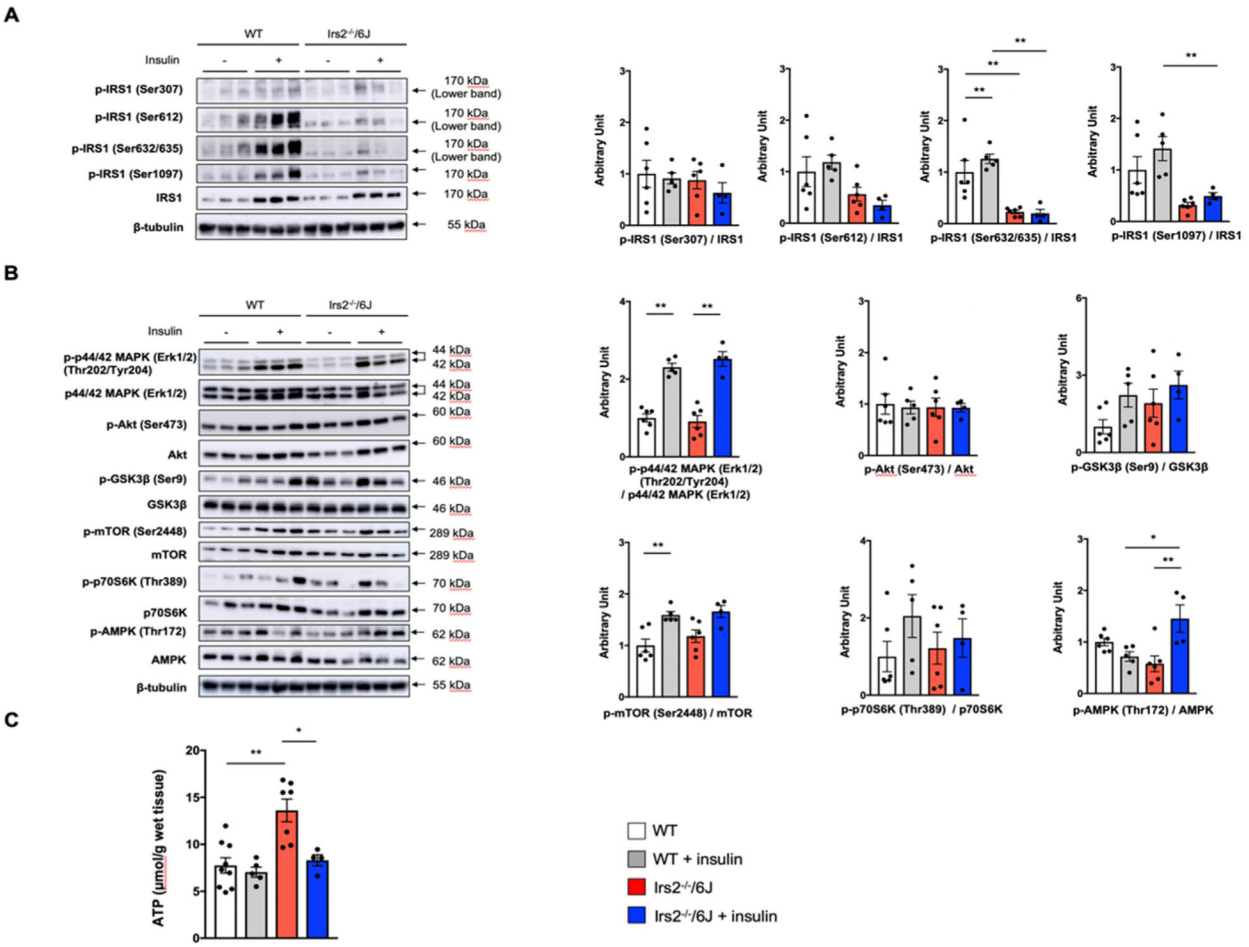

Fig. 3. Abnormal activation of metabolic energy sensor in the hippocampus of young adult male *Irs2*$^{-/-}$/6J mice.

Western blot analysis: (A) IRS1 phosphorylation at the mouse Ser307 (mSer307), mSer612, mSer632/635, and mSer1097 sites and total IRS1 and β-tubulin content in the hippocampi of the WT and *Irs2*$^{-/-}$/6J mice fasted and treated with insulin (16–20 weeks, n = 4–6 per group). An arrow indicates their respective bands (lower band) in (A). The phosphorylation of IRS1 at their respective sites were normalized to total protein content; (B) the phosphorylation levels of p44/42 MAPK Erk1/2 Thr202/Tyr204, Akt Ser473, GSK3β Ser9, mTOR Ser2448, p70S6K Thr389, and AMPK Thr172 and the respective total protein levels and β-tubulin in the hippocampi of both mice fasted and treated with insulin (16–20 weeks, n = 4–6 per group). The phosphorylation of their respective molecules was normalized to their respective total protein content. (C) The levels of ATP in the hippocampi of both mice fasted and treated with insulin (16–20 weeks, n = 7–9 or 4–5 per group). Results are presented as mean ± standard error of the mean (SEM), *$p < 0.05$; **$p < 0.01$.

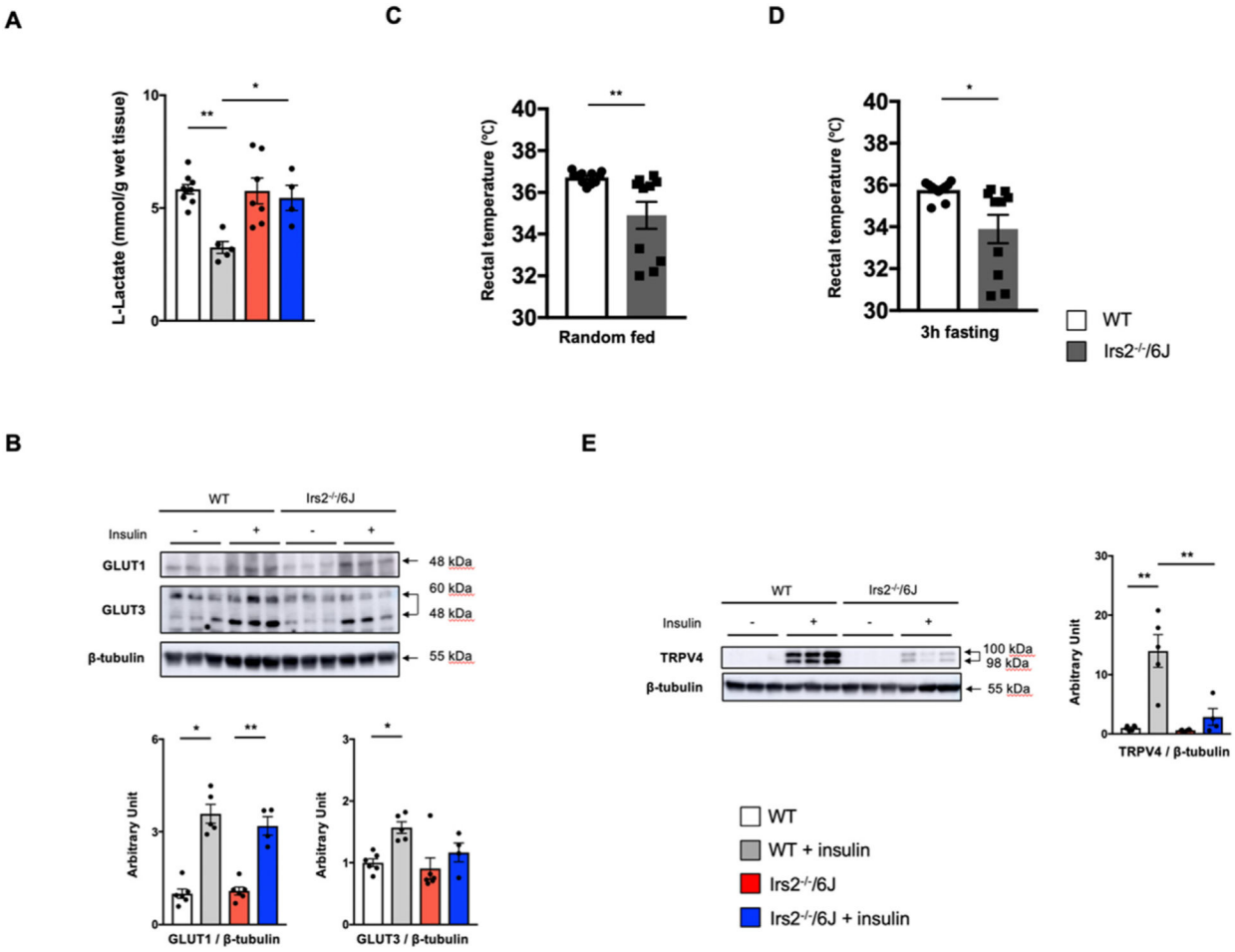

Fig. 4. Downregulation of body temperature and its related factor in the brain of young adult male *Irs2*$^{-/-}$/6J mice.

(A) Levels of L-Lactate in the hippocampi of the WT and *Irs2*$^{-/-}$/6J mice fasted and treated with insulin (16–20 weeks, n = 7–9 or 4–5 per group). (B) Western blot analysis of GLUT1, GLUT3, and β-tubulin in the hippocampi of both mice fasted and treated with insulin (16–20 weeks, n = 7–9 or 4–5 per group). The GLUT1 and GLUT3 protein levels were normalized to the β-tubulin protein content. (C) Randomly fed rectal temperature and (D) 3h-fasting rectal temperature in the WT and *Irs2*$^{-/-}$/6J mice (13–16 weeks, n = 10–11 per group). (E) Western blot analysis of the levels of TRPV4 and β-tubulin in the hippocampi of both mice fasted and treated with insulin (16–20 weeks, n = 4–6 per group). The TRPV4 protein levels were normalized to the β-tubulin protein contents. Results are presented as mean ± standard error of the mean (SEM); *$p < 0.05$; **$p < 0.01$.