Inactivation of the adrenergic receptor β_2 disrupts glucose homeostasis in mice

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Abstract

Three types of beta adrenergic receptors (AR β_1 –3) mediate the sympathetic activation of brown adipose tissue (BAT), the key thermogenic site for mice which is also present in adult humans. In this study, we evaluated adaptive thermogenesis and metabolic profile of a mouse with *Ar β_2* knockout (AR β_2 KO). At room temperature, AR β_2 KO mice have normal core temperature and, upon acute cold exposure (4 °C for 4 h), AR β_2 KO mice accelerate energy expenditure normally and attempt to maintain body temperature. AR β_2 KO mice also exhibited normal interscapular BAT thermal profiles during a 30-min infusion of norepinephrine or dobutamine, possibly due to marked elevation of interscapular BAT (iBAT) and of *Ar β_1* , and *Ar β_3* mRNA levels. In addition, AR β_2 KO mice exhibit similar body weight, adiposity, fasting plasma glucose, cholesterol, and triglycerides when compared with WT controls, but exhibit marked fasting hyperinsulinemia and elevation in hepatic *Pepck* (*Pck1*) mRNA levels. The animals were fed a high-fat diet (40% fat) for 6 weeks, AR β_2 KO mice doubled their caloric intake, accelerated energy expenditure, and induced *Ucp1* expression in a manner similar to WT controls, exhibiting a similar body weight gain and increase in the size of white adipocytes to the WT controls. However, AR β_2 KO mice maintain fasting hyperglycemia as compared with WT controls despite very elevated insulin levels, but similar degrees of liver steatosis and hyperlipidemia. In conclusion, inactivation of the AR β_2 KO pathway preserves cold- and diet-induced adaptive thermogenesis but disrupts glucose homeostasis possibly by accelerating hepatic glucose production

Key Words

- β -adrenergic receptors
- adaptive thermogenesis
- brown adipose tissue (BAT)
- obesity

and insulin secretion. Feeding on a high-fat diet worsens the metabolic imbalance, with significant fasting hyperglycemia but similar liver structure and lipid profile to the WT controls.

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Introduction

Brown adipose tissue (BAT) is an important site of adaptive thermogenesis, preserving thermal homeostasis in response to cold exposure in both rodents and humans (Cannon & Nedergaard 2004, Nedergaard & Cannon 2010), as well as dissipating caloric excess in rodents in response to feeding with a high caloric diet (Rothwell & Stock 1979, Vosselman *et al.* 2013). Thus, understanding of the mechanisms regulating BAT activity may help in development of new strategies to accelerate energy expenditure and address the current high prevalence of obesity.

In mice, BAT develops during the 3-day window E16.5–18.5 (Hall *et al.* 2010) and after birth can be activated by the sympathetic nervous system (SNS) through stimulation of β -adrenergic receptors (AR β) and cAMP production (Cannon & Nedergaard 2004). In BAT, cAMP signaling not only induces the expression of genes involved in the thermogenic capacity of the tissue but also promotes lipolysis and activates the mitochondrial uncoupling protein-1 (Ucp1) that leads to heat production. The importance of this pathway is illustrated by the fact that mice in which all three AR β isoforms have been inactivated exhibit increased susceptibility to cold exposure as well as diet-induced obesity (Bachman *et al.* 2002, Jimenez *et al.* 2002).

The AR β s are members of a family of three G-protein-coupled receptors, i.e. β_1 , β_2 , and β_3 , that are distributed throughout the body and play their metabolic roles by controlling glucose homeostasis, lipolysis, and insulin secretion (Gardner & Shoback 2007). All three AR β isoforms are expressed in BAT (Collins & Surwit 2001) and our recent studies have indicated that the AR β_1 is key for cold- and diet-induced BAT thermogenesis as the AR β_1 KO mice develop hypothermia when exposed to cold and obesity due to being fed a high-fat diet (HFD) (Ueta *et al.* 2012). In contrast, the roles played by AR β_2 and AR β_3 are less clear. Inactivation of the *Ar β_3* gene seems to be very well tolerated *in vivo* given that mice with AR β_3 KO exhibit only a modest increase in body fat, have normal basal metabolic rate, and respond normally to cold exposure (Susulic *et al.* 1995), which could be due to upregulation of AR β_1 (Atgie *et al.* 1997).

There is great interest in the study of the AR β_2 pathway in humans. For example, activation of the AR β_2 pathway with salbutamol accelerates energy expenditure, lipolysis, and fat oxidation without affecting glucose oxidation in humans (Hoeks *et al.* 2003). In fact, a specific genetic variation in the *AR β_2* gene is associated with blunted *in vivo* AR β -mediated lipolysis and fat oxidation during AR β stimulation (Jocken *et al.* 2007). In addition, polymorphisms of the *AR β_2* gene are frequent in obese humans (Large *et al.* 1997, Takenaka *et al.* 2012), but studies on associations between these polymorphisms and body weight and composition, glucose tolerance, and insulin sensitivity have produced mixed results (Echwald *et al.* 1998, Kortner *et al.* 1999, Oberkofler *et al.* 2000, Prior *et al.* 2011).

Despite great interest in AR β_2 in humans, available animal models indicate only a minor metabolic role for AR β_2 (Rohrer *et al.* 1999). For example, it is known that AR β_2 KO mice have lower body weight and smaller epididymal fat pads (Chruscinski *et al.* 1999), but their metabolic rate remains unaffected when compared with WT control animals (Rohrer *et al.* 1999). However, AR β_2 KO mice have not been studied under conditions in which thermogenesis is activated. Thus, herein we looked at different metabolic parameters in AR β_2 KO animals that were acutely exposed to cold or fed with a HFD. We found that AR β_2 KO mice expressed increased liver phosphoenolpyruvate carboxykinase (*Pepck*) mRNA levels and displayed marked hyperinsulinemia, which on a HFD also results in increased fasting blood glucose. However, these animals attempt to maintain their body temperature when exposed to cold and exhibit normal catecholamine-stimulated interscapular BAT (iBAT) thermal response. When fed a HFD, AR β_2 KO mice exhibited similar susceptibility to diet-induced obesity when compared with WT controls. Notably, there was a dramatic increase in the expression of both *Ar β_1* and *Ar β_3* mRNAs in the BAT of AR β_2 KO mice, possibly explaining the lack of a major thermogenic phenotype.

Materials and methods

Animals

Approximately 60-day-old Friend virus B (FVB) male AR β_2 KO mice and WT FVB controls were studied, following an animal protocol approved by the Institutional Committee on Animal Research at the Center of Biological Sciences and Health-University Presbyterian Mackenzie. Each experiment was repeated two or three times on different sets of animals. As indicated, mice were kept on a chow diet (1.8 Cal/g) and water was available for drinking *ad libitum* in a room maintained at 25 °C. Food intake and body weight were measured daily. Acute cold exposure was performed in conscious mice housed individually in cages with no bedding in a cold room maintained at 4 °C (Eletrolab, São Paulo, SP, USA) for up to 4 h. Colonic temperature was measured hourly using a 1 mm wide rectal probe (Y4000, YSI, Yellow Springs, OH, USA) as previously described (de Jesus *et al.* 2001). Some animals were fed a HFD (5.44 Cal/g; 40% lipid; Rhoister, Sao Paulo, BR, USA) for 5 weeks.

Resting oxygen consumption (VO₂)

VO₂ was measured in an open-circuit respirometer system (O2-10, Sable System, Las Vegas, NV, USA) as described previously (Curcio *et al.* 1999). For cold exposure, mice were exposed to different temperatures (5, 15, 25, 30, 32, 34, and 36 °C) and VO₂ was measured for 1 h at each temperature. The experiments were conducted between 1100 and 1900 h. Data were collected and analyzed using the Sable Systems' software and results expressed as ml O₂/min per g BW and ml O₂/min as indicated. When at room temperature (25 °C), measurements were obtained over 30 min periods, in the afternoon (1400–1800 h) in animals that had access to food *ad libitum*.

Intraperitoneal glucose tolerance test and insulin tolerance test

Overnight fasting blood glucose was measured weekly in all animals by sampling the tail vein and using a glucose analyzer (LifeScan, Inc., Milipitas, CA, USA). For the glucose tolerance test (GTT), animals were fasted overnight and received glucose (2 g/kg) injected i.p. between 0900 and 1000 h. Blood samples were collected from the tail vein at the indicated times after the glucose load and assessed for glucose analysis. For the insulin tolerance

test (ITT), food was removed 6 h before the experiment, which was carried out between 1400 and 1500 h. Blood samples were collected from the tail vein at the indicated times after injection of insulin (0.5 U/kg; i.p.) and glycemia was immediately determined using the glucose analyzer. At the end of the experimental period, blood samples were collected by cardiac puncture and assessed for insulin levels by ELISA (Marschner *et al.* 1974).

iBAT thermal response to norepinephrine or dobutamine infusion

This was determined under anesthesia as described previously (Ribeiro *et al.* 2001, Bianco *et al.* 2014). Briefly, mice were anesthetized with urethane (560 mg/kg, i.p.) and chloralose (38 mg/kg, i.p.) in the morning on the day of the experiment and a polyethylene (P-50) cannula was inserted into the left jugular vein. iBAT temperatures (°C) were measured using a precalibrated thermistor probe (YSI 427; Yellow Springs Instrument Co., Yellow Springs, OH, USA) surgically placed under the iBAT pad. Temperature was monitored until it reached a stable baseline (approximately 10 min), followed by infusion of norepinephrine (NE) (2 mg/ml) or the selective β_1 agonist dobutamine (DB) (600 μ g/ml) (Aikawa *et al.* 1996, Huang *et al.* 1998) through the left jugular vein using an infusion pump (model 2274, Harvard Apparatus, Holliston, MA, USA) at a rate of 0.643 μ l/min for 30 min.

mRNA analysis

iBAT and liver were dissected and total RNA extracted using Trizol (Life Technologies, Inc.), according to the manufacturer's instructions, and quantified by spectrophotometry. For the reverse transcriptase reaction, 1.0 μ g total RNA was used in the ImProm-II Reverse Transcription System for RT-PCR (Promega), on a Robocycler thermocycler (Stratagene, La Jolla, CA, USA). Based on the reaction efficiency, about 120 ng of cDNA was used for amplification. Quantitative real-time PCR (RT-qPCR) was carried out using an IQ SYBR Green PCR kit (Bio-Rad) on an iCycler thermal cycler (Bio-Rad). The housekeeping gene cyclophilin A was used as an internal reference. Primer sequences were available upon request. The cycle conditions were as follows: 5 min at 94 °C, 30 s at 94 °C, 30 s at 58 °C, and 45 s at 72 °C for 50 cycles followed by the melting curve protocol to verify the specificity of amplicon generation. Gene expression was determined by the $\Delta\Delta$ Ct method, as described previously (Christoffolete *et al.* 2004).

Western blot

iBAT was also processed for mitochondrial isolation. Mitochondrial proteins were then size-fractionated by 12% SDS-PAGE and probed with α -UCP1 (Santa Cruz Biotechnology).

Blood chemistry

Total serum cholesterol and triglycerides were assessed via enzymatic methods using a commercial kit (Roche Molecular Biochemicals).

Histology

After dissection, tissues were immersed in buffered formaldehyde solution and fixed for 24 h. Paraffin-embedded tissues were sectioned and processed as described for staining with hematoxylin–eosin or Masson's trichrome (Kerr *et al.* 1995). The cellular area of the white adipocytes was measured by analyzing pictures of at least 40 adipocytes per animal taken at 200 $\times$ magnification.

Statistical analysis

The statistical analyses were done by ANOVA followed by the Student–Newman–Keuls post-test when $P < 0.05$ and by analysis of covariance (ANCOVA) followed by the Bonferroni post-test as indicated.

Results

Thermogenic profile of AR β_2 KO animals in response to cold or infusion with catecholamines

Mice acclimatized to room temperature were exposed to acute cold temperature at 4 °C and core body temperatures

were measured hourly for up to 4 h. Both WT controls and AR β_2 KO mice maintained their core temperature (Fig. 1A) by progressively accelerating resting energy expenditure (REE) (Fig. 1B). The iBAT thermal response to infusion with a defined dose of catecholamines provides information about the BAT thermogenic capacity (Ribeiro *et al.* 2001). In WT animals acclimatized to room temperature, infusion of NE over 30 min resulted in an increase of approximately 2.0 °C in iBAT temperature, similar to what was observed in the AR β_2 KO animals (Fig. 1C).

The possibility that AR β_2 KO animals compensate for the disruption of the AR β_2 pathway by activating other β -adrenergic pathways was tested by measuring mRNA levels for Ar β_1 and Ar β_3 in the BAT of AR β_2 KO animals. Indeed, mRNA levels for both ARs were greatly elevated in the AR β_2 KO BAT (Fig. 1D). However, the iBAT thermal response to the selective β_1 agonist DB was similar in WT and in β_2 KO animals (Fig. 1E), indicating that the AR β_2 KO BAT maintains its thermogenic capacity, which can be maximally stimulated via the AR β_1 pathway.

Susceptibility of AR β_2 KO mice to diet-induced obesity

Adult AR β_2 KO mice exhibited similar caloric intake (Fig. 2A) and body weight (Fig. 2B). Mice were then fed a HFD and monitored for 8 weeks, these animals exhibited increased caloric intake with no differences between AR β_2 KO and WT controls (Fig. 2A). At the same time, all animals gained weight and those on the HFD gained approximately twice as much, again with no differences between AR β_2 KO and WT controls (Fig. 2B and C). REE as expressed in relation to BW (VO_2 ml/min/g BW) was not different in AR β_2 KO when compared with WT controls (Fig. 2D) even when data were plotted as a function of BW (Supplementary Figure 1A, see section on supplementary data given at the end of this article) as suggested previously (Tschop *et al.* 2012). At the

Figure 1

Effects of the lack of β_2 adrenergic receptor on cold-induced thermogenesis. (A) Central body temperature during cold exposure (4 °C) for 3 h of WT and ARKO β_2 ; (B) oxygen consumption during exposure of WT and ARKO β_2 mice to decreasing temperatures; (C) brown adipose tissue thermogenic response to norepinephrine (NE) infusion of WT and ARKO β_2 mice; (D) gene

expression of β_1 and β_3 adrenergic receptors in mice kept on a chow or HFD in BAT (cross hatched (black and white), WT; white, AR β_2 KO); (E) brown adipose tissue thermogenic response during infusion of DB of WT and ARKO β_2 mice. Entries are mean $\pm$ s.e.m. of five animals per group.

Figure 2

Effects of the lack of β_2 adrenergic receptor on diet-induced thermogenesis. (A) Caloric intake calculated based on daily food consumption; (B) initial BW; (C) variances of BW (Δ BW) are shown; (D) VO_2 assessed during 30 min at the end of the experiment expressed relative to BW (VO_2 ml/min/g per BW); (E) VO_2 assessed during a period of 30 min at

the end of the experiment expressed as absolute values (VO_2 ml/min) and statistically controlled for the effects of body weight (covariant); (F) estimated individual epididymal adipocytes area; 40 cells for each group were analyzed; (G) UCP1 expression determined by western blot. Values are mean $\pm$ S.E.M. of five animals per group.

same time, REE was accelerated by 8 weeks of HFD in both WT controls and AR β_2 KO animals, albeit less so in the latter group (Fig. 2D). These differences between the two groups of animals remained even when data were plotted as a function of BW (Supplementary Figure 1B, C and D). When the same data are expressed in terms of absolute values (VO_2 ml/min) and statistically controlled for the effects of body weight (covariant) then the HFD-induced acceleration in REE was only observed in the WT controls (Fig. 2E). The epididymal white adipocyte area (Fig. 2F) and *Ucp1* levels also behaved similarly in both AR β_2 KO and WT controls (Fig. 2G), except that AR β_2 KO mice on a chow diet exhibited significantly higher *Ucp1* levels when compared with WT controls.

Glucose and insulin tolerance in AR β_2 KO mice

Fasting blood glucose levels were monitored weekly during the 7-week experimental period (Fig. 3A) and no differences were observed until the end of the first week of HFD. From that point on, all animals on the HFD

developed fasting hyperglycemia that was transiently more intense in the WT controls but that during weeks 5–7 became more pronounced in the AR β_2 KO animals (Fig. 3A). Upon further investigation during week 8 of the experiment, all animals fed a HFD exhibited an apparent greater intolerance to glucose as assessed by the area under the curve of the GTT (Fig. 3B and C). However, when blood glucose was expressed at each time-point as a percentage of basal values, only the WT controls fed with the HFD exhibited mild glucose intolerance, whereas no differences were found in the AR β_2 KO animals (Fig. 3D and E). Sensitivity to insulin was similar in AR β_2 KO and WT controls, with both groups exhibiting a slight but significant decrease in the AUC when kept on a HFD (Fig. 3F and G). As expected, during week 7, insulin levels after overnight fasting were elevated in WT controls placed on the HFD (Fig. 3H). At the same time, insulin levels were dramatically elevated in the AR β_2 KO animals regardless of the type of diet (Fig. 3H). The elevated fasting glucose – not associated with decreased insulin sensitivity or glucose

Figure 3

Effects of the lack of β_2 adrenergic receptor on glucose and insulin tolerance of mice kept on a chow diet or on a HFD. (A) Fasting blood glucose levels during the 7-week experimental period; (B and C) blood glucose levels before and after i.p. administration of 2 g/kg glucose (GTT) expressed as absolute values and (D and E) expressed at each time-point as

a percentage of basal values; (F and G) blood glucose levels after i.p. injection of 0.5 U/kg insulin (ITT); (H) insulin serum levels after overnight fasting and (I) *Pepck* mRNA levels in liver of WT and AR β_2 KO animals kept on chow or high-fat diets. (A) $P < 0.05$ and $**P < 0.01$ vs WT HFD. Entries are mean $\pm$ S.E.M. of five animals per group.

intolerance – indicates that hepatic glucose production is accelerated in the AR β_2 KO animals. This is in fact supported by the observation that liver *Pepck* (*Pck1*) mRNA levels are doubled in the AR β_2 KO animals kept on chow diet and further elevated in both strains by HFD (Fig. 3I).

Liver steatosis in AR β_2 KO mice

Feeding on a HFD caused liver steatosis indistinctly in both groups of animals, i.e. AR β_2 KO and WT animals (Fig. 4).

Cholesterol and triglyceride plasma levels in AR β_2 KO mice

No differences in serum cholesterol and triglycerides were observed between AR β_2 KO and WT animals. Both parameters increased similarly in both groups of animals after they were fed a HFD.

Discussion

It is well recognized that AR β s play a significant role in metabolic control and energy homeostasis, including

adaptive thermogenesis (Bachman *et al.* 2002). The results of the present studies indicate that inactivation of the AR β_2 pathway interferes with glucose homeostasis, most probably by accelerating hepatic neoglucogenesis and glucose production, which is largely neutralized by a marked elevation in insulin levels. However, feeding the animals with a HFD stresses the systems further, beyond what can be compensated for by an elevation of insulin secretion, resulting in fasting hyperglycemia and relative glucose intolerance.

Although data obtained in the AR β_1 KO mouse indicated that the AR β_1 pathway is important for BAT thermogenesis (Ueta *et al.* 2012), the present studies indicate that the role played by AR β_2 in BAT thermogenesis is much less significant and possibly redundant. Animals with inactivation of the AR β_2 pathway exhibit a normal baseline metabolic profile and respond indistinguishably from WT controls when exposed to cold, or treated with infusions of adrenergic agonists, but exhibit lesser acceleration of REE when fed a HFD. Alternatively, it is conceivable that the AR β_2 pathway plays an important role in BAT thermogenesis and the apparent lack of a

Figure 4

Liver steatosis in $AR\beta_2$ KO mice fed HFD. Liver sections stained by H&E; magnification is $180\times$.

thermogenic phenotype is explained by the overexpression of $AR\beta_1$ and $AR\beta_3$ in BAT. Thus, $AR\beta_2$ KO mice provide a suitable model for studying the interrelationships between the $AR\beta_2$ pathway and glucose homeostasis, which is also observed in humans. However, the minor role played by the $AR\beta_2$ pathway in BAT thermogenesis is in contrast to the wealth of literature supporting a role for $AR\beta_2$ in energy homeostasis and adaptive thermogenesis in humans (Hoeks *et al.* 2003). If confirmed, this could indicate important differences in the mechanisms involved in energy homeostasis between the two species.

$AR\beta_2$ activation promotes dilation of smooth muscle in different organs and structures but also plays an important role in energy homeostasis by slowing peristaltic movements and gastrointestinal secretion, as well as promoting lipolysis, insulin secretion, gluconeogenesis, and glycogenolysis, thus leading to overall lower levels of blood glucose (Gardner & Shoback 2007). Therefore, it is notable that $AR\beta_2$ KO animals exhibit a marked elevation in serum insulin when on a chow diet and, when on a HFD they exhibit greater disruption in glucose homeostasis, with fasting hyperglycemia and relative glucose intolerance. Such findings are reminiscent of those observed in humans exposed to long-term overfeeding. Subjects with the $AR\beta_2$ Gln27Gln polymorphism or the 3.7/3.4 kb BanI variant experienced a greater increase in insulin resistance than Glu27Glu/Gln27Glu subjects (Ukkola &

Bouchard 2001). Furthermore, in obese postmenopausal women, different $AR\beta_2$ haplotypes were associated with glucose intolerance, and thus may mediate insulin action, glucose tolerance, and potentially risk for type 2 diabetes mellitus (Prior *et al.* 2011).

Previous studies with β agonists-induced activation of glucose uptake in astrocytes and skeletal muscle cells indicated a key role of β_2 in SNS-mediated glucose uptake, allowing for synergy between SNS and insulin signaling (Nevzorova *et al.* 2006, Catus *et al.* 2011). This is in contrast to the present observation that overall sensitivity to insulin is not modified in the $AR\beta_2$ KO mice (Fig. 3F and G). At the same time, $AR\beta_1$ and $AR\beta_3$ have been recognized as key mediators in glucose uptake (Nikami *et al.* 1996, Chernogubova *et al.* 2004) and our data supports these findings. In addition, while metoprolol, a β_1 antagonist, worsens insulin resistance, carvedilol, a β_2 and α_1 antagonist, decreases insulin resistance in type 2 diabetes patients (Phillips *et al.* 2008).

The role of the $AR\beta_2$ pathway in adaptive thermogenesis was tested during cold exposure or feeding on a HFD, two processes that involve multiple organs and tissues, e.g. hypothalamus, skeletal muscle, liver, and white adipose tissue and BAT. The latter is where most heat is generated in response to SNS stimulation (Enerback *et al.* 1997). Thus, the present data do not support a major role for $AR\beta_2$ in cold- or diet-induced thermogenesis.

First, the AR β_2 KO animals maintained their core temperature and increased VO₂ during acute cold exposure (4 h), indicating normal BAT function. That this is a process primarily dependent on BAT is illustrated by the dramatic hypothermia developed during a similar time frame of cold exposure in UCP-1KO mice (Enerback *et al.* 1997). That the BAT thermogenesis is largely preserved in the AR β_2 KO animals is further verified by the observation that their iBAT responds normally to NE infusion. Notably, the only biochemical difference observed in the AR β_2 KO BAT was a slightly elevated baseline mitochondrial UCP1 content, a finding of undetermined significance. Second, when fed a HFD the overall thermogenic response seems to be preserved in the AR β_2 KO animals given that caloric intake, weight gained, and the size of white fat depots behaved similarly to those of WT controls. This occurred despite lesser acceleration of the REE, at least as assessed through a limited 1 h-diurnal VO₂ study.

AR β_2 is present (Sell *et al.* 2004) and fully functional (Atgie *et al.* 1997) in the murine BAT. Although its inactivation would be expected to result in a detectable phenotype, previous studies have indicated that overexpression of other *Ar β* isoforms could provide a compensatory mechanism. For example, increased signaling via the AR β_1 or AR β_2 pathways was thought to explain the resistance to diet-induced obesity of AR β_3 KO mice (Atgie *et al.* 1997). In fact, there is marked overexpression of *Ar β_1* and *Ar β_3* mRNA in the BAT of AR β_2 KO animals. However, when treated with infusions of the AR β_1 -selective agonist, DB, AR β_2 KO animals exhibited a similar iBAT thermal response, indicating that the AR β_1 is not hyper-responsive to stimulation. Although this could reflect limitations in other steps of the BAT thermogenic pathway, at face value these data strengthen the argument that AR β_2 plays only a minor role if any in BAT thermogenesis.

The present data indicate that AR β_2 does not play a significant role in lipid metabolism, given that AR β_2 KO mice exhibit similar hyperlipidemia when fed a HFD. In addition, AR β_2 KO animals fed a HFD developed liver steatosis indistinguishable from WT controls. These data are relevant considering that AR β blockers have been implicated in the increase in triglycerides and cholesterol plasma levels of hypertensive patients (Sarafidis & Bakris 2006, Bell *et al.* 2009). Also, patients with the AR β_2 -Glu27 variant have hypertriglyceridemia with elevated cholesterol levels (Iaccarino *et al.* 2005). However, long-term treatment with oral broxaterol, a selective AR β_2 agonist, has minimal metabolic effects on lipid metabolism (Petraglia *et al.* 1990). Thus, it is not clear that AR β_2 KO

mice would be useful for modelling the role played by the AR β_2 pathway in lipid metabolism in humans.

In conclusion, the AR β_2 KO pathway does play a significant role in glucose homeostasis, probably by accelerating hepatic glucose production that is largely neutralized by increased insulin secretion. Fasting hyperglycemia only occurs if the system is further stressed, for example by feeding a HFD. The study of the AR β_2 KO mouse also indicated that the AR β_2 pathway plays a less significant role in the acute BAT thermogenic response and overall diet-induced thermogenesis.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/JOE-13-0526>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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