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Ablation of the GNB3 gene in mice does not affect body weight, metabolism or blood pressure, but causes bradycardia

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Abstract

G protein $\beta 3$ (G $\beta 3$) is an isoform of heterotrimeric G protein β subunits involved in transducing G protein coupled receptor (GPCR) signaling. Polymorphisms in G $\beta 3$ (GNB3) are associated with many human disorders (e.g. hypertension, diabetes and obesity) but the role of GNB3 in these pathogenesis remains unclear. Here, G $\beta 3$ -null mice (GNB3^{-/-}) were characterized to determine how G $\beta 3$ functions to regulate blood pressure, body weight and metabolism. We found G $\beta 3$ expression restricted to limited types of tissues, including the retina, several regions of brain and heart ventricles. G $\beta 3$ -deficient mice were normal as judged by body weight gain by age or by feeding with high-fat diet (HFD); glucose tolerance and insulin sensitivity; baseline blood pressure and angiotensin II infusion-induced hypertension. During tail-cuff blood pressure measurements, however, G $\beta 3$ -null mice had slower heart rates (~450 vs ~500 beats/min). This bradycardia was not observed in isolated and perfused G $\beta 3$ -null mouse hearts. Moreover, mouse hearts isolated from GNB3^{-/-} and controls responded equivalently to muscarinic receptor- and β -adrenergic receptor-stimulated bradycardia and tachycardia, respectively. Since no difference was seen in isolated hearts, G $\beta 3$ is unlikely to be involved directly in the GPCR signaling activity that controls heart pacemaker activity. These results demonstrate that although G $\beta 3$ appears dispensable in mice for regulation of blood pressure, body weight and metabolic features associated with obesity and diabetes, G $\beta 3$ may regulate heart rate.

Keywords

Heterotrimeric G protein; GNB3; GPCR; signalling; polymorphism; blood pressure; metabolism; obesity; diabetes

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1. Introduction

Heterotrimeric G proteins transmit signals from G protein coupled receptors (GPCRs) to regulate diverse physiological processes, including metabolism, cardiovascular function and immune response [1, 2]. Dysregulation of GPCRs and G proteins is linked to various diseases, including hypertension, obesity, and diabetes [3-8]. G proteins are composed of $G\alpha$, $G\beta$ and $G\gamma$ subunits; $G\beta$ and $G\gamma$ form an obligate dimer and play a central role in G protein signaling [9]. $G\beta$ and $G\gamma$ subunits each assume multiple isoforms, and are encoded by five distinct $G\beta$ genes ($G\beta1$, $G\beta2$, $G\beta3$, $G\beta4$ and $G\beta5$) and 12 $G\gamma$ genes, respectively. The majority of $G\beta$ and $G\gamma$ isoforms form a functional dimer when expressed in a heterologous expression system; however, some evidence suggests specific $G\beta$ and $G\gamma$ isoforms perform distinct functions *in vivo*. For example, mice $G\beta1^{-/-}$ mice are embryonic lethal and display neural tube defects [10], while mice with a $G\beta5$ deficiency are viable but their vision and brain functions are impaired [11-13], suggesting the function of these two subunits is non-redundant *in vivo*. Likewise, $G\gamma1$, $G\gamma3$, $G\gamma5$, $G\gamma7$ and $G\gamma13$ knockout mouse phenotypes are different, suggesting the various $G\beta$ and $G\gamma$ couplings perform distinct functions [14-20]. Nevertheless, our understanding of the functional specificity of $G\beta$ and $G\gamma$ subunits is far from complete, and the role of other $G\beta$ and $G\gamma$ subunits have not yet been characterized *in vivo* by genetic manipulations in mice.

Of the five $G\beta$ genes, $G\beta3$ is unique in that a number of genetic polymorphisms are known [21-24]. C825T is one of the most well characterized $G\beta3$ polymorphisms and is associated with a shorter, spliced variant, $G\beta3s$ [24]. This polymorphism was initially identified in patients with essential hypertension; and later population-based association studies linked $G\beta3s$ to diverse disorders, including obesity, diabetes, depression, and tumors [24-27]. For many of these studies, however, the results are inconsistent and in many cases contradictory [28-33].

The initial characterization of $G\beta3s$ by Siffert's group suggested $G\beta3s$ was a gain-of-function mutant with enhanced activity both in promoting GPCR-mediated $G\alpha$ activation and stimulating $G\gamma$ effectors [22, 23]. We and others recently demonstrated, however, that $G\beta3s$ is an unstable protein and unable to form a functional dimer with various $G\gamma$ subunits [34, 35]. These findings suggest any pathological effects associated with the $G\beta3$ C825T polymorphism are likely the consequence of $G\beta3$ inactivation. To understand the physiological function of $G\beta3$ *in vivo*, we characterized $G\beta3$ expression patterns and investigated the role of $G\beta3$ in regulating blood pressure, body weight and metabolism in $G\beta3$ -null mice. Our results indicate that $G\beta3$ is expressed in limited types of tissues and does not play a major role in regulating blood pressure, body weight and metabolic features associated with obesity and diabetes, but may contribute to signaling involved in the control of heart rate.

2. Materials and methods

Mice

Heterozygous $GNB3^{+/-}$ C57BL/6N mice were produced by the Knockout Mouse Project at the University of California, Davis. The entire coding region of $GNB3$ gene was replaced

by the bacterial β -galactosidase (*lacZ*) gene through homologous recombination (Fig. 1A). As reported [36], we found it difficult to breed homozygous mice, so experimental mice were bred from heterozygous mice. Mouse genotyping was performed by PCR using the following oligonucleotides: Primer 1: 5'-GCAGCCTCTGTTCCACATACACTTCA-3'; primer 2: 5'-CTAGGAGCCACATCCATAGTCATGGG-3'; primer 3: 5'-TGGAAGTGAAGGCTAGAGGAAGAG-3'. Amplification from the wild type genome with primers 1 and 2 produced a 358 bp product and the *GNB3*^{-/-} genome with primers 3 and 2 produced a 458 bp product (Fig. 1B).

Mice were housed in a standard 12:12 dark-light cycle with access to standard chow and water ad libitum, unless specified. Mice were weighed weekly 1 week after birth. In some studies, mice were fed with either normal chow (13.5% fat; LabDiet) or HFD (45% fat; D12492, Research Diets) ad libitum beginning at 10 weeks of age for up to 4 weeks, and body weight was monitored weekly. Animal experiments were approved by the University of Iowa Animal Care and Use Committee.

RNA isolation and quantitative PCR (q-PCR)

Total RNA was isolated from various tissues using TRIzol (Life Technologies) and treated with DNaseI (New England Biolab) according to the manufacturers' instructions. First strand cDNA was synthesized using iScript cDNA synthesis kit (Bio-Rad); and q-PCR samples were run in a 25 μ l reaction (iQ SYBR green supermix, BioRad) using CFX96 q-PCR system (Bio-Rad). All samples were run in triplicate and normalized to the mouse housekeeping gene *PPIB* (peptidylprolyl isomerase B) using the C_t method [37]. Primers for mouse *GNB1*, *GNB3* and *PPIB* are as follows: *GNB1* forward: 5'-TCTGGGAATTATGTGGCCTG-3'; *GNB1* reverse: TGATTGTCATCCAGGAACCG; *GNB3* forward: 5'-TCTACAACCTCAAATCCCGC-3'; *GNB3* reverse: 5'-TCTCAATGTCCCACAAGGC-3'; *PPIB* forward: 5'-GGAGATGGCACAGGAGGAA-3'; *PPIB* reverse: 5'-GCCCCGTAGTGCTTCAGCTT-3'.

Detection of *lacZ* activity

LacZ activity was detected in whole mounts as described previously [38]. Briefly, 11.5 d embryonic offspring from breeding between *GNB3*^{+/-} mice were dissected in cold phosphate buffered saline (PBS) and fixed in 4% paraformaldehyde/PBS for 1 hr at 4 °C. Embryos were washed three times in rinse buffer (5 mM EGTA, 0.01% deoxycholate, 0.02% NP40, 2 mM MgCl₂, in PBS) at room temperature, and immersed in staining buffer (mM K₃[Fe(CN)₆], 5 mM K₄[Fe(CN)₆], 1 mg/ml X-gal, in rinse buffer) for up to 48 hours at 30 °C. For genotyping, the yolk sac surrounding the embryos was dissected and subjected to genomic DNA preparation and PCR.

To determine *lacZ* activity in mouse tissues, 20 μ g of cell lysates were prepared from various tissues (using lysis buffer: 0.1 M Tris-HCl, pH 7.8, 0.5% Triton X-100) and incubated with o-nitrophenyl- β -D-galactopyranoside (0.4 mg/ml) in 300 μ l of buffer (1 mM MgCl₂, 45 mM β -mercapoethanol, 0.1 M sodium phosphate, pH 7.5) at 37 °C for 24 hr [39]. The reaction was stopped by adding Na₂CO₃ to a final concentration of 0.625 M. The

optical density of the solution was read at a 420 nm wavelength, using a Biotek Synergy 4 microplate reader.

Resting metabolic rate

Resting metabolic rate was measured as previously described [40-42]. Briefly, O₂ and CO₂ exchange of mice during sleep at thermoneutrality (30°C) were measured using S-3A/II and CD-3A analyzers, respectively (AEI). Mass flow of effluent air was measured (Sensiron) and STP-corrected. Data were recorded and analyzed using a PowerLab (ADInstruments) and associated Chart software. Heat was estimated using the formula derived from Lusk [43];

$$Heat = VO_2 \times (1.232 \times RER + 3.815)$$

where *Heat* is calculated as kcal/hr, VO₂ represents STP-corrected rate of oxygen consumption (in mL/min), and the respiratory exchange ratio, RER, represents the ratio of carbon dioxide production to oxygen consumption (unitless).

Food intake and motor activity

The food intake and physical activity of mice were monitored by a Comprehensive Lab Animal Monitoring System (CLAMS; Columbus Instrument). For these experiments, male animals with matching body weight were individually housed for 4 days in metabolic cages and fed normal chow or a HFD. The amount and frequency of food intake were recorded and motor activity was monitored by infrared beam interruption.

Glucose tolerance and insulin sensitivity tests

Intraperitoneal glucose tolerance and insulin sensitivity tests were performed using 12 to 14 week-old male mice. Mice first fasted for 5 hr and their baseline glucose level was measured from a drop of blood from a tail snip wound and Accu-chek active glucometers and test strips (Roche Diagnostics). Mice were then injected intraperitoneally with 100 µl of glucose (2 g/kg lean mass of mice) or human insulin (Eli Lilly and Company; 0.5 U/kg body mass of mice), and blood glucose levels monitored at 15, 30, 45, 60, 90, 120 and 180 min post-injection [44].

Blood pressure and heart rate measurement

Blood pressure and heart rate were measured using a BP-2000 tail-cuff blood pressure analysis system (Visitech Systems) as previously described [45]. Briefly, 14 to 16 week-old mice were acclimated to warmed restraint boxes daily for 2 weeks. Once acclimated, 30 measurements of systolic blood pressure and heart rate were averaged from each animal daily for 2 weeks to assess baseline blood pressure. In some cases, Angiotensin II (AngII; Sigma Aldrich; 1 µg/kg/min) was delivered to subsets of mice by osmotic minipump (model 1004, Alzet). After osmotic minipump implantation, pressures and heart rates were recorded daily for 10 days to assess drug effects.

Langendorff-Perfusion and Electrocardiography (ECG) measurements

ECG recording from Langendorff-perfused hearts was performed as described [46]. Hearts were excised from 12 to 14 week-old male mice and mounted on a modified Langendorff apparatus (HSE-HA perfusion systems, Harvard Apparatus). Retrograde aortic perfusion was performed at a constant pressure of 80 mm Hg with Krebs-Henseleit buffer (25 mM NaHCO₃, 118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄, 1.2 mM NaH₂PO₄, 2.5 mM CaCl₂, 0.5 mM Na-EDTA, and 15 mM glucose, pH 7.4) oxygenated with 95% O₂ and 5% CO₂. After the heart was allowed to stabilize for 30 min, ECG recording of the perfused heart was continuously recorded with Ag⁺-AgCl electrodes. The hearts were perfused with 1 μM carbachol (CCh) for 10 min, washed for 20 min with buffer, followed by 5 nM isoproterenol for 10 min and then 1 μM carbachol (CCh) for another 5 min in the continued presence of isoproterenol.

Western blotting

Proteins were extracted from isolated tissues using modified RIPA buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% deoxycholate, 1% NP-40) containing various protease inhibitors, and quantified by the Bradford assay [47]. 50-100 μg proteins were resolved on 10% SDS-PAGE and probed with specific antibodies. Immunoblots were analyzed by an Odyssey infrared imaging system (LI-COR Biosciences).

Statistical analyses

Data are expressed as the mean ± SEM and analyzed by *t* test or ANOVA. Comparison between groups was analyzed by 2-tailed Student's *t* test or multifactor ANOVA, with differences considered significant at *P* < 0.05.

3 Results

3.1. Gβ3 is expressed at low levels in restricted tissues

Various tissues have been shown to express Gβ3, as detected by Northern blot, Western blot and immunohistochemistry; however, in many cases, the probe specificity was not well characterized [36, 48, 49]. Since we know of no commercially available antibody that cleanly detects Gβ3, to examine the Gβ3 expression pattern, we took advantage of the GNB3^{-/-} mice, in which the entire Gβ3 coding region is replaced with a β-galactosidase gene. This β-galactosidase gene is under the control of the endogenous Gβ3 promoter, so its expression can serve as a surrogate marker for Gβ3-expressing cells. Whole-mount analysis of E11.5 embryos, by lacZ staining, did not detect lacZ activity in any tissue, suggesting Gβ3 is not significantly expressed during early embryonic development (Fig. 2A). Similar results were found with whole-mount staining of isolated tissues from adult mice (not shown). A significant level of lacZ activity was, however, detected in tissue lysates prepared from the retina but not from other major organs (Fig. 2B), indicating the retina is the major site of Gβ3 expression. Consistent with these findings, qRT-PCR analysis showed that a majority of tissues, including cerebellum (1%), hypothalamus (6%), hippocampus (1.5%), brain stem (0.2%), ventricle (4%), spleen (2%), kidney (1%), stomach (1%), large intestine (0.2%), small intestine (0.2%), bladder (1%), white adipose tissue (WAT; 1.2%), only

expressed a small percentage of G β 3 transcripts in the retina (Fig. 2C, D). This is in contrast to G β 1, which was expressed at a more comparable level in the retina and a majority of tissues examined (Fig. 2C). Moreover, in most tissues tested, G β 1 transcripts were expressed at a level over 100 fold higher than G β 3 transcripts (data not shown). G β 3 transcripts were undetectable in many tissues, such as cortex, olfactory bulb, liver, lung, skeletal muscle, brown fat tissue (BAT) and large and small arteries and veins (Fig. 2B, C, D).

3.2. G β 3 deficiency does not affect body weight, metabolism and metabolic features associated with obesity and diabetes

G β 3 polymorphisms have been associated with obesity and diabetes [26, 50], and G β 3 duplication implicated in a childhood obesity syndrome [51]. To determine if G β 3 plays a role in body weight gain, we measured mouse body weight weekly in the control and G β 3-null mice, beginning from two weeks after birth. No significant difference was observed in body mass between the wild-type and G β 3^{-/-} mice, regardless of sex (Fig. 3A and not shown). When fed a HFD, both the wild-type and G β 3^{-/-} mice gained weight at a similar rate (~4.1 g/week) (Fig. 3B), and their major organs had equivalent mass after mice had been fed with the HFD for three weeks (Fig. 3C). Over 7 consecutive days in metabolism cages, G β 3^{-/-} and wild-type mice consumed a similar amount of food, showed similar resting metabolic rates (as characterized by heat production) and had similar physical activity, regardless of whether they were fed with normal chow or HFD (Fig. 4A, B, C, and not shown). G β 3^{-/-} and wild-type mice also had the same level of fasting glucose, and exhibited the same glucose tolerance and insulin sensitivity (Fig. 5A, B). Together, these data indicate that in mice, G β 3 does not play a major role in regulating body mass, resting metabolism or metabolic features associated with obesity and diabetes.

3.3. G β 3 deficiency did not affect blood pressure control but caused bradycardia

We next determined whether G β 3 deficiency affects blood pressure and heart rate, since G β 3 polymorphisms have been associated with hypertension and arrhythmia [24, 52]. The baseline systolic blood pressure was not significantly different between G β 3^{-/-} and wild-type mice, but the heart rates of G β 3^{-/-} mice were about 50 beats/min lower than that of the control (~450 vs. 500 beats/min, $p < 0.05$; Fig 6A, C). Angiotensin II infusion similarly increased systolic blood pressure in the wild-type and G β 3^{-/-} mice (Fig. 6B), but did not alter the bradycardia observed in the G β 3^{-/-} mice (Fig. 6D). Interestingly, this bradycardia was not present in isolated perfused heart preparations of G β 3^{-/-} mice (Fig. 7A, B). Moreover, the isolated perfused hearts of G β 3^{-/-} mice were indistinguishable from those of controls in their responses to carbachol- and isoproterenol-induced bradycardia and tachycardia, respectively (Fig. 7A, B). These findings suggest the bradycardia in G β 3-null mice is probably not triggered by intrinsic defects of G protein signaling in the heart. In supporting this notion, the activity of AKT and ERKs in the atrium and ventricle was not significantly different between G β 3^{-/-} and wild-type mice (Fig. 8A, B). Moreover, the expression levels of G α subunits, including G α i, G α s and G α 11, in the atrium were not altered by G β 3 deficiency (Fig. 8A).

4. Discussion

Extensive studies have been conducted to elucidate the linkage of G β 3 polymorphisms with multiple disorders, but little is known about the physiological functions of G β 3 *in vivo*. In this study, we evaluated, for the first time, the effect of G β 3 ablation on body weight, metabolism and blood pressure in mice. Moreover, we presented the first examination of endogenous expression of G β 3 in various tissues. Overall, our results did not find a significant role for G β 3 in regulating body weight, metabolic features associated with obesity and diabetes and blood pressure. The dispensable role of G β 3 in regulating these functions may owe to its relatively low level of expression in restricted tissues, as compared to other G β isoforms. For example, G β 3 was detected only in the retina when lacZ activity was used as a surrogate marker for its expression. Although G β 3 transcripts were detected in a number of tissues when expression was measured by a more sensitive assay (qRT-PCR), G β 3 transcript levels were at least 100 fold lower than G β 1 levels. Moreover, G β 3 expression was negligible in the major tissues important for metabolic and blood pressure regulation (i.e., liver, fat and blood vessels). Thus, the signaling function of G proteins in various tissues may be carried out primarily by other G β isoforms. Nevertheless, we cannot exclude the possibility that G β 3 is expressed in a subset of cells in particular tissues and participates in G protein-mediated signaling cascades. Indeed, consistent with our findings that the retina is the major site of G β 3 expression, G β 3 was recently shown to be the major G β isoform expressed in retinal cones and ON bipolar cells and was shown to be critical for efficient light responses [36, 53]. Recent reports also indicate that transgenic mice carrying an extra copy of the G β 3 gene gained more weight than wild-type mice, suggesting that under a pathological level of G β 3 overexpression [51], G β 3 may be important for weight-control signaling. We also cannot rule out the possibility that some G β 3 functions may be compensated for by other G β isoforms, in response to lifelong loss of G β 3 expression. Moreover, the tail-cuff plethysmography method may not be sensitive enough to detect subtle changes in blood pressure caused by G β 3 deletion [54]. Nevertheless, given that both the control and G β 3-null mice responded equally well to a large blood pressure increase induced by angiotensin II infusion, it is unlikely that G β 3 plays a major role in blood pressure control, as previously proposed [24].

Interestingly, when measured by the tail-cuff method, G β 3-null mice exhibited bradycardia, which persists under Angiotensin II infusion-induced hypertension. Heart rate is normally determined by the pacemaker activity of sinoatrial node (SA node) located in the right atrium, and modified by autonomic regulation [55]. Activation of sympathetic systems increases heart rate by releasing catecholamine on to β -adrenergic receptors located on SA nodal cells [56]. In contrast, acetylcholine released from the vagus binds to muscarinic M $_2$ receptors in SA nodal cells, which activates Gi/o proteins to release G γ . G γ binds to and activates G protein-coupled, inwardly rectifying K $^+$ channels, leading to membrane hyperpolarization and bradycardia [57]. Thus, ablation of G β 3 may decrease heart rate by altering the intrinsic automaticity of SA nodal cells, and/or autonomic control of SA nodal cells via β -adrenergic receptors and/or muscarinic M $_2$ receptors. We found, however, that in the isolated perfused heart, neither the baseline heart rate nor a carbachol- or isoproterenol-induced decrease or increase in heart rate was affected by G β 3 deficiency. Moreover, G β 3

deficiency neither affected the activity of GPCR effectors, such as AKT and ERKs, nor the expression of $G\alpha$ subunits in the atrium or ventricle. These results suggest $G\beta 3$ functions outside of the heart to control heart rate. These findings are in contrast with previous suggestion that $G\beta 3$ is directly involved in regulating atrial inwardly rectifying K^+ channels [58], but consistent with the data that $G\beta 3$ is not expressed in the atrium, where SA nodal cells are located. Nevertheless, it remains to be determined how exactly $G\beta 3$ contributes to heart rate regulation. Since $G\beta 3$ is expressed in several regions of brain, it could potentially participate in autonomic regulation of heart rate via GPCR-mediated signaling cascades in the central nervous system.

The initial discovery by Siffert et al. that hypertensive patients often harbored the $G\beta 3$ C825T polymorphism has stimulated extensive followup studies to determine the association of $G\beta 3$ polymorphisms with diverse human diseases [24, 26]. Many studies attempted to attribute the effect of $G\beta 3$ C825T polymorphism to a shorter form of $G\beta 3$, $G\beta 3s$, which presumably has enhanced signaling activity, but the results of those studies have been inconsistent [22, 23, 34]. Very few of these studies, however, actually examined the expression and functionality of $G\beta 3s$. We recently characterized the biochemical properties of $G\beta 3s$ and provided unambiguous evidence that unlike $G\beta 3$, $G\beta 3s$ is a functionally inactive protein that fails to form a dimer with diverse $G\gamma$ subunits [35]. These findings indicate that any pathological effects of $G\beta 3$ C825T polymorphism likely result from downregulation of $G\beta 3$ function. In this study, our findings that $G\beta 3$ ablation in mice does not affect weight, metabolism or blood pressure raises the question of whether any human disorders associated with the $G\beta 3$ C825T polymorphism can be attributed to the function of $G\beta 3$. Although we cannot rule out the possibility that $G\beta 3$ may play a different role in human versus mouse, the function of $G\beta 3$ bears further examination.

5. Conclusions

In conclusion, our results demonstrate that $G\beta 3$ does not play a significant role in regulating body weight, metabolism or blood pressure, but may contribute to the signaling that regulates heart rate.

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Abbreviations

AngII	angiotensin II
GPCR	G protein-coupled receptor

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Highlights

- G β 3 is expressed in restricted tissues
- Ablation of G β 3 in mice does not affect body weight, resting metabolisms, metabolic features associated with obesity and diabetes, and blood pressure
- G β 3-null mice exhibit bradycardia

Figure 1. Generation and genotyping of G β 3^{-/-} mice

A, schematic representation of the construct used to generate G β 3^{-/-} mice. The location of the primers used to distinguish wild-type (p1 and p2) and G β 3^{-/-} (p3 and p2) mice is indicated. B, representative images of genotyping results by PCR. +/+, wild-type; +/-, heterozygous; -/-, homozygous G β 3 knockout.

Figure 2. G β 3 expression in various tissues

A, lacZ staining of whole-mount embryos (E11.5). *B*, lacZ activity in tissue lysates prepared from 3 month-old mice with the indicated genotypes. *C-D*, expression of G β 3 and G β 1 transcripts in various tissues by qRT-PCR analysis. Data were normalized with PPIB transcripts and are expressed as percentage of G β 3 transcript levels in the retina. N=3.

Figure 3. Gβ3 deficiency does not affect body weight

A, measurement of mouse weight (male) by age under normal chow. WT n=4-30; Gβ3^{-/-} n=4-30. B, measurement of mouse weight under normal (NC) or high fat diet (HFD). WT NC n=8; WT HFD n=6; Gβ3^{-/-} NC n=8; Gβ3^{-/-} HFD n=7. C, tissue masses of WT and Gβ3^{-/-} mice. WT HFD n=4; Gβ3^{-/-} HFD n=5.

Figure 4. G β 3 ablation does not affect food intake, energy expenditure and physical activity
A, food intake. N=10. **B**, resting metabolic rate at thermoneutrality. N=4. **C**, physical activity. WT n=5; G β 3^{-/-} n=6.

Figure 5. G β 3 deficiency does not affect glucose tolerance and insulin sensitivity

A, glucose tolerance test. WT n=6; G β 3^{-/-} n=8. B, insulin sensitivity test. WT n=8; G β 3^{-/-} n=8.

Figure 6. Gβ3 ablation does not affect blood pressure control but causes bradycardia
Systolic blood pressure (A) and heart rate (B) in control mice (control) and mice infused with angiotensin II ((AngII) for 10 days. The number of mice used in the studies is indicated in the graphs. *, ** $p < 0.05$ and 0.01 versus control, respectively.

Figure 7. Gβ3 deficiency does not affect heart rates in isolated perfused heart

A, Heart rates were determined from ECGs of isolated hearts at basal condition (basal), in the presence of 1 μ M carbachol (CCh), 5 nM isoproterenol (Iso), or 5 nM isoproterenol plus 1 μ M carbachol (Iso+CCh). WT n=5; Gβ3^{-/-} n=4. *, **, *** p<0.05, 0.01 and 0.001 *versus* baseline, respectively; # p<0.05 *versus* Iso. B, representative traces of ECGs are shown.

Figure 8. Gβ3 deficiency does not affect GPCR signaling or Gβ subunit expression

Immunoblot analysis of the levels of phosphorylated AKT473 and ERKs (A, B), or Gαi, Gαs, and Gα11 (A) in the atrium (A) and ventricle (B) of the wild-type (WT) and GNB3^{-/-} mice. Mortalin was used as a loading control. Each lane represents extracts from individual mouse. Quantification of protein expression (normalized by Mortalin) in the atrium is also shown (A).