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Sevoflurane Impairs Insulin Secretion and Tissue-Specific Glucose Uptake *In Vivo*

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Abstract: The use of anaesthetics severely influences substrate metabolism. This poses challenges for patients in clinical settings and for the use of animals in diabetes research. Sevoflurane can affect regulation of glucose homoeostasis at several steps, but the tissue-specific response remains to be determined. The aim of the study was to investigate the pharmacological effect of sevoflurane anaesthesia on glucose homoeostasis during hyperinsulinaemic clamp conditions, the gold standard method for assessment of whole-body insulin sensitivity. Conscious mice (n = 6) and mice under sevoflurane anaesthesia (n = 8) underwent a hyperinsulinaemic clamp where constant infusion of insulin and donor blood was administered during variable glucose infusion to maintain isoglycaemia. 2-[1-^{14}C]-deoxy-D-glucose was infused to determine tissue-specific uptake of glucose in adipose tissue, heart, brain and skeletal muscle. Sevoflurane anaesthesia severely impaired insulin-stimulated whole-body glucose uptake demonstrated by a 50% lower glucose infusion rate (GIR). This was associated with decreased glucose uptake in brain, soleus, triceps and gastrocnemius muscles in sevoflurane-anaesthetized mice compared to conscious mice. Plasma-free fatty acids (FFA), a potent inducer of insulin resistance, increased by 42% in mice during sevoflurane anaesthesia. In addition, insulin secretion from pancreatic β-cell was lower in fasted, anaesthetized mice. Sevoflurane anaesthesia impairs insulin secretion, induces insulin resistance in mice and reduces glucose uptake in non-insulin-sensitive tissue like the brain. The underlying mechanisms may involve sevoflurane-induced mobilization of FFA.

Hyperglycaemia is associated with poor clinical outcomes during surgery in critically ill patients [1]. Elevated blood glucose levels are associated with increased infection rates after general surgery [2] and correlate positively with mortality after myocardial infarction and stroke [3]. For all hospitalized patients, those with hyperglycaemia have a higher mortality rate than those with normal glucose levels [4].

Hyperglycaemia is a well-known side effect to several types of anaesthetic drugs [5]. Sevoflurane, a commonly used anaesthetic [6], impairs glucose tolerance after IV glucose tolerance test (IVGTT) and intraperitoneal glucose tolerance test (IPGTT) in rodents [7]. This may involve impaired insulin secretion from the pancreas [8], and reduced pharmacodynamics effects of insulin manifested as hepatic insulin resistance. Thus, in a canine model, sevoflurane anaesthesia suppressed insulin-mediated glucose disposal by 20% compared to conscious dogs during a hyperinsulinaemic clamp [8]. Less is known about the effects of anaesthesia on tissues important for glucose disposal. In human beings, skeletal muscle is thought to account for most of the glucose disposal after an oral glucose load [9], and insulin resistance in skeletal muscle is prominent in type 2 diabetes [10]. Hepatic glucose homoeostasis shifts from glycogenolysis/gluconeogenesis to glycogen synthesis by insulin signalling [11]. During general anaesthetic procedures in human beings, there is an extensive inhibition of substrate oxidation in muscle mitochondria [12], which may affect glucose disposal. The brain has a resting energy expenditure of 240 kcal/kg/day from glucose, and ketones serve as an alternative energy source [13]. Unlike muscle, substrate utilization rates in the brain are known to be unaffected by anaesthesia [14].

Understanding the tissue-specific effects of sevoflurane on substrate metabolism may allow for prevention of complications to anaesthesia induced by hyperglycaemia. Combining the hyperinsulinaemic isoglycaemic clamp with a radioactive-labelled glucose tracer allows for determination of tissue-specific glucose uptake [15]. Therefore, these techniques were applied in conscious, unrestrained and sevoflurane-anaesthetized C57BL/6JBomTac mice to test the hypothesis that sevoflurane affects skeletal muscle insulin resistance. The primary outcome was glucose infusion rate, and tissue-specific glucose uptake was analysed to specify alterations in glucose uptake.

Methods

Animals. The experiment was conducted following the guidelines of The Animal Experiments Inspectorate in Denmark, license number: 2012-15-2934-152. Mice were housed in a regulated environment. Room temperature was maintained at 22°C, a 12-hr light–dark cycle (7:00 a.m. and 7:00 p.m.) was used, and mice were allowed free

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access to a standard chow diet containing 24% protein, 5% fat, 6% ash, 3% fibre, 8% water and 54% nitrogen-free extract and water. Wild-type male C57BL/6JBomTac mice (Taconic Biosciences, Borup, Denmark) were 14–16 weeks of age. Fourteen (14/31) mice entered the experiment on a weekly basis for two consecutive weeks. Six mice, 27.8 ± 0.5 g, were investigated while awake, and eight mice, 29.5 ± 0.7 g, were anaesthetized with 2.0% sevoflurane during the hyperinsulinaemic isoglycaemic clamp experiments. Fourteen (14/31) matched mice acted as blood donors to ensure adequate red blood cells during the clamp procedure. Two mice experienced blocked catheters and were excluded from the study, and one mouse died during post-operation recovery. In total, 31 mice were used for this study.

Surgical procedures for hyperinsulinaemic isoglycaemic clamp experiments. The clamp procedure was performed in mice 6–7 days after catheterization of the carotid artery and the jugular vein as described previously [16]. In short, catheters were prepared from silastic and polyethylene tubing and mice were anaesthetized with isoflurane (2.5% for induction, 1.5% for maintenance) during surgery. Carprofen (10 mg/kg; Rimadyl, Pfizer, New York, USA) was administered pre-operatively for analgesia. Lidocaine (7 mg/kg; Xylocain, AstraZeneca, Gothenburg, Sweden) was administered for local analgesia during surgery. Seventeen mice underwent surgical procedure, and fourteen had patent catheters after recovery. The catheters were locked with heparinized saline (200 U/mL) and the animals recovered for 6–7 days. The inclusion criteria following surgery were a weight loss of less than 10% and a haematocrit of more than 35% by post-op day 6–7, and no visible signs of pain or infection. All mice fulfilled these criteria. Details of the surgical procedures, hyperinsulinaemic euglycaemic clamp and the determination of plasma and tissue radioactivity are described elsewhere [16].

Hyperinsulinaemic isoglycaemic clamp. Five-hour fasted mice underwent hyperinsulinaemic isoglycaemic clamp for 120 min. to obtain steady-state conditions. Glucose was infused at variable rates to maintain basal blood glucose (isoglycaemic) levels throughout the clamp procedure, as previously described [17]. The hyperinsulinaemic glucose clamp in conscious mice used in the study has previously been demonstrated to involve a minimum of physical activity and stress during blood sampling [18]. On the day of the clamp experiment, at 7 a.m. ($t = -300$ min.), food was removed and the mice were placed in a clean cage with bedding and nesting material. The mice had free access to water. A divider was placed in the cage restricting the accessible area to ~20 × 20 cm. At $t = -180$ min., the catheters were flushed with heparinized saline (10 U/mL), and the mice were connected to the infusion lines. Sevoflurane was administrated to animals in the anaesthesia group (2.5% for induction, 2.0% for maintenance during the clamp experiment) and placed on a heat pad to maintain body temperature (−120 min.). The conscious group of mice was unrestrained in their cage during the entire clamp experiment. A tracer equilibration period was started in both groups at $t = -90$ min. by infusion of a 1.2 μCi priming dose of [3-^{3}H] glucose (Perkin Elmer, San Jose, CA, USA). This was followed by a constant 0.04 μCi/min. infusion of [3-^{3}H] glucose for 90 min. Blood samples for the assessment of haematocrit levels as well as basal glucose and insulin levels, and turnover rates were collected at $t = -15$ min. (50 μL) and −5 min. (100 μL), respectively. The clamp was initiated at $t = 0$ min. with continuous infusions of human insulin (4 mU/kg/min.; Actrapid, Novo Nordisk, Denmark) and red blood cells from a donor mouse to compensate for the blood loss due to repeated sampling (5 μL/min. of 50% RBC in 10 U/mL heparinized saline). Entirety of the plasma component was removed to control for any circulating insulin or glucose from donor blood. Insulin was diluted in succinylated gelatin (Gelofusine® B. Braun Melsungen AG, Berlin, Germany). Blood glucose was measured every 10 min. (Bayer Contour, Pittsburgh, PA, USA), and isoglycaemic blood glucose levels (7 mmol/L) were maintained in the conscious group of mice by adjusting a variable infusion of 50% glucose containing 0.06 μCi/μL of [3-^{3}H] glucose tracer. The anaesthetized group was maintained at their fasted blood glucose level (isoglycaemic ~10 mmol/L). Steady-state was achieved at $t = 80$ min. to $t = 120$ min., when the glucose infusion rate equals glucose uptake by all the tissues in the mouse. At steady-state, the GIR, measured in mg/kg/min., is a measure of whole-body insulin sensitivity. During steady-state, blood samples (50–100 μL) were collected at 10-min. intervals and processed for determination of [3-^{3}H] glucose-specific activity. At $t = 120$ min., a 12 μCi bolus of 2-[1-^{14}C]-deoxy-D-glucose (Perkin Elmer) was injected, and blood samples were collected at $t = 122$, 125, 135, 145 and 155 min., respectively. These samples were processed for determination of 2-[1-^{14}C]-deoxy-D-glucose-specific activity. After the final blood sample, mice were euthanized with a lethal dose of pentobarbital. Tissues were collected and snap-frozen in liquid nitrogen. The tissues were processed for determination of 2-[1-^{14}C]-deoxy-D-glucose-specific activity to calculate tissue-specific glucose uptake. Plasma samples were processed for determination of endogenous glucose production (glucose rate of appearance (endoR_a) and rate of disappearance (R_d)) as previously described [15].

Sample processing and calculations. Plasma samples were deproteinized with $Ba(OH)_2$ and $ZnSO_4$, and aliquots of each supernatant were transferred to 2 scintillation vials as described previously [16]. Glucose turnover rates (rate of appearance (R_a) rate of disappearance (R_d), endoRa, glycolysis) were calculated as previously described [18]. Tissues were homogenized in ice-cold lysis buffer (pH 7.4, 10% glycerol, 1% IGEPAL, 50 mmol/L Hepes, 150 mmol/L NaCl, 10 mmol/L NaF, 1 mmol/L EDTA, 1 mmol/L EGTA, 20 mmol/L sodium pyrophosphate, 2 mmol/L sodium orthovanadate, 1 mmol/L sodium pyrophosphate, 5 mmol/L nicotinamide, 4 μmol/L thiamet G and protease inhibitors (Sigma Aldrich, Brøndbyvester, Denmark). Aliquots of each crude homogenate were transferred to two 2-mL tubes and deproteinized with perchloric acid or $Ba(OH)_2 + ZnSO_4$. Supernatants were transferred to scintillation vials and counted to determine 2-[1-^{14}C]-deoxy-D-glucose content. Tissue-specific glucose uptake rates were calculated as described previously [19]. Only four mice in each group received 2-[1-^{14}C]-deoxy--glucose. The remaining six mice were omitted due to technical failure in tracer administration.

Glucose, insulin and FFA levels in plasma. Human and mouse insulin concentrations in plasma samples, basal (−15 min.) and steady-state (80–120 min.) were analysed on the MSD platform (Meso Scale Diagnostics, Rockville, MD, USA) with mouse and human insulin ELISA (Mercodia, Uppsala, Sverige). During the clamp, blood glucose levels were measured by a handheld glucometer (Bayer, Contour). We verified all the plasma glucose with a Glucose Assay Kit (Abcam, Cambridge, UK). FFA quantification of basal (−15 min.) plasma was performed with NEFA-HR (Wako, Neuss, Germany).

Statistical analysis. All data are presented as mean ± S.E.M. Unpaired *t*-test and multiple *t*-test analyses were made in GraphPad Prism 7. All time-dependent analyses were evaluated by two-way repeated-measures ANOVA. Statistical significance was set at $p < 0.05$. Primary end-point for this study is GIR and based on previous mouse studies where standard deviation was 0.15 in steady-state GIR in conscious mice. G*Power was used to calculate sample size for two groups with five repeated measurements. Given alpha, power and effect size were used: $\alpha = 0.05$, effect size = 0.5, power = 0.80. A total sample size of eight, n = 4 in each group, was found to be a sufficient group size. We included an additional four mice in each group in the study to allow for exclusion of mice for technical reasons such as failed surgeries and blocked catheters.

Results

Sevoflurane anaesthesia induces hyperglycaemia and decreases whole-body insulin sensitivity.

Fasting blood glucose levels in anaesthetized mice were 89% higher prior to insulin infusion, and the animals remained at a higher blood glucose throughout the clamp (fig. 1A). After 80 min. of insulin infusion, the blood glucose in the anaesthetized mice decreased by 25%, but remained 60% higher than awake mice throughout steady-state (80–120 min.). Despite hyperglycaemia, the glucose infusion rate (fig. 1B) was lower in all sevoflurane-anaesthetized mice compared to conscious mice, demonstrating whole-body insulin resistance. Basal insulin levels were not statistically different between the two groups (fig. 1C) despite a 60% increase in blood glucose levels. During clamp conditions, all mice received weight-adjusted constant insulin infusion and this led to similar plasma insulin levels during steady-state in the two groups (fig. 1D).

Tissue-specific effects on glucose uptake during sevoflurane anaesthesia.

The reduced glucose infusion rate in the sevoflurane group was associated with decreased insulin-stimulated glucose uptake in soleus, gastrocnemious and triceps muscles (fig. 2). The effects of sevoflurane were not confined to classical insulin-regulated tissues, like skeletal muscle. Most notably, we found that brain tissue also displayed a >50% reduction in glucose uptake. A tendency to reduced insulin-stimulated glucose uptake during sevoflurane was found in all other tissues except subcutaneous fat, but these tendencies did not reach statistical significance. Because anaesthetized mice had significantly lower GIR, we calculated whole-body glycolysis and glucose storage under steady-state conditions and found a ~50% reduction of both in sevoflurane-treated mice (fig. 3A). In contrast, endogenous glucose production (EGP) was not affected by sevoflurane prior to the insulin clamp conditions (fig. 3B). During steady-state, the insulin-mediated suppression of EGP was 40% in conscious mice and 80% in sevoflurane-anaesthetized mice, but the difference between groups did not reach statistical significance.

Lipid metabolism is alternated in anaesthetized mice.

A main effect of insulin in adipose tissue is suppression of lipolysis and reduced release of non-esterified fatty acids. We therefore investigated whether sevoflurane anaesthesia affected circulating levels of FFA. In anaesthetized mice, plasma FFA levels were ~40% higher than in conscious mice prior to insulin infusion, indicating increased lipolysis (fig. 3C).

Discussion

Sevoflurane is the most commonly administered fast-acting inhalational anaesthetic in the United States [6]. Sevoflurane is advantageous due to solubility in blood and tissues and a low frequency of respiratory irritation [20], circulatory stimulation

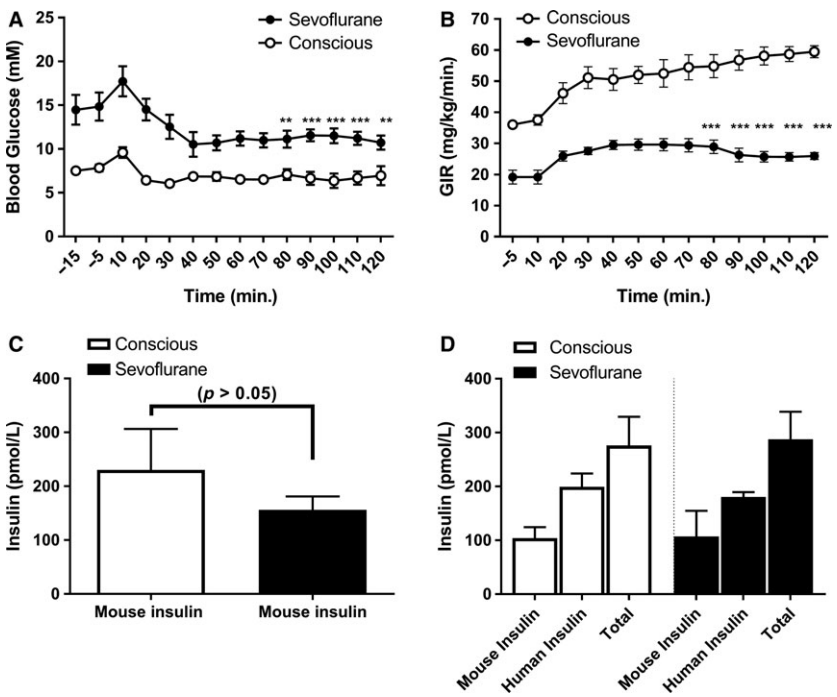

Fig. 1. Hyperinsulinaemic isoglycaemic clamp. Blood glucose levels during the hyperinsulinaemic euglycaemic clamp conditions. Comparisons by two-way ANOVA, $^{**}p < 0.01$, $^{***}p < 0.001$. (B) Glucose infusion rate during the clamp. Comparisons two-way ANOVA, $^{***}p < 0.001$. (C) Basal plasma insulin levels in 5-hr fasted mice, non-significant (ns) ($p > 0.05$) by the unpaired Student's *t*-test. (D) No difference between mouse insulin, human insulin and total plasma insulin levels during steady-state (SS), 80–120 min. In all panels, data are mean ± S.E.M. n = 5–8 in sevoflurane group and n = 4–6 in conscious group.

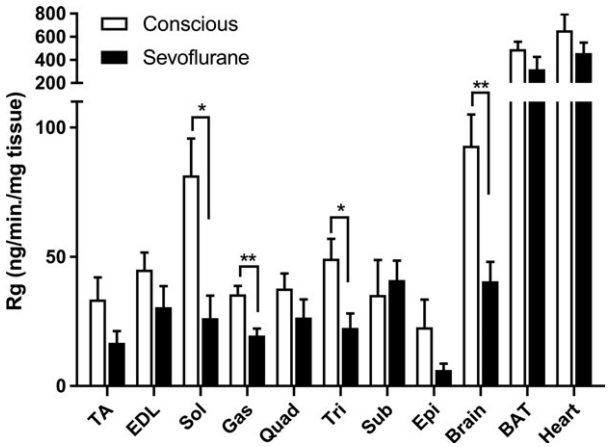

Fig. 2. Tissue-specific glucose uptake. Tissue-specific glucose metabolic index (Rg). Tibialis anterior (TA), extensor digitorum longus (EDL), soleus (Sol), gastrocnemius (Gas), quadriceps (Quad), triceps (Tri), subdermal subcutaneous fat (Sub), epididymal fat (Epi), brain, brown adipose tissue (BAT) and heart tissue. *$p < 0.05$, **$p < 0.01$ by unpaired Student's *t*-test N = 4 in each group.

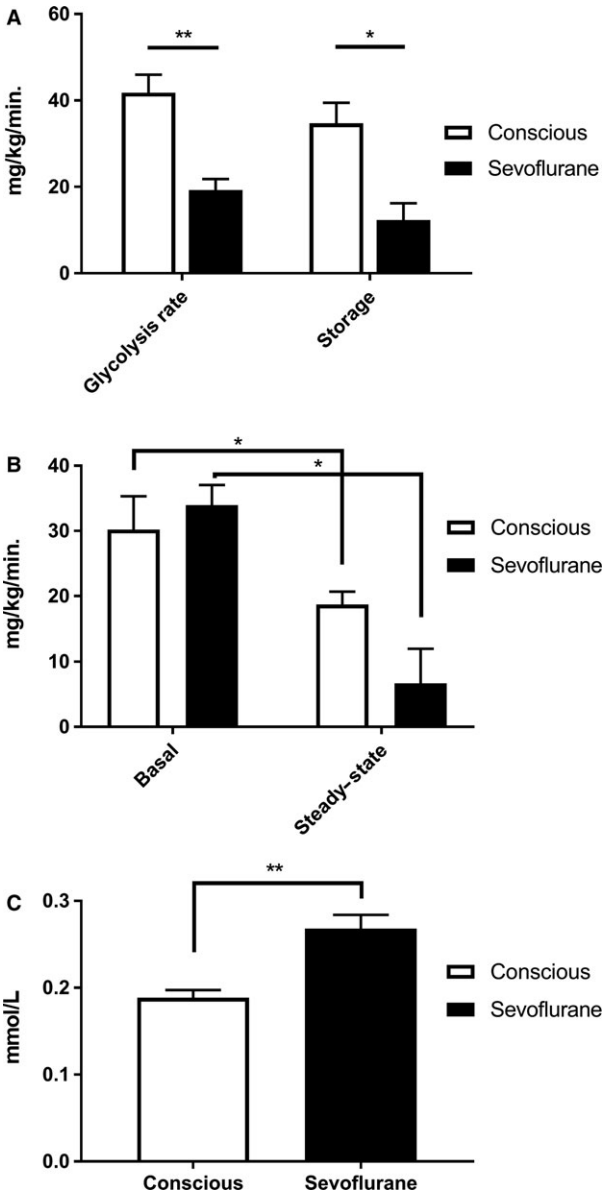

Fig. 3. Steady-state plasma measurements. (A) Glycolysis rate and glucose storage. n = 8–4. Unpaired Student's *t*-test (B) endogenous glucose production, calculated from [3-^{3}H] glucose tracer in plasma, two-way ANOVA. (C) FFA in plasma prior to insulin infusion. Unpaired Student's *t*-test. *$p < 0.05$, **$p < 0.01$.

[21] and hepatotoxicity [22]. However, among the side effects of sevoflurane is impairment of glucose homoeostasis, and further insight into the underlying mechanisms behind this side effect is still unclear. Clinical data suggest that sevoflurane anaesthesia impairs glucose tolerance through lowering of acute insulin response and rates of glucose disappearance [23]. Using gold standard methods to assess insulin sensitivity in animals, this study demonstrates that sevoflurane severely affects glucose homoeostasis by (i) inducing insulin resistance in skeletal muscle, (ii) reducing glucose uptake in the brain and (iii) inhibiting insulin release from the β-cell. These data exemplify the complexity of using anaesthetics and indicates that multiple mechanisms are involved in the interaction between insulin and sevoflurane.

Insulin levels in sevoflurane-anaesthetized mice were markedly lower despite high blood glucose levels. This observation aligns with results from human beings where insulin secretion is impaired during anaesthesia [23]. In insulin-producing β-cells, adenosine triphosphate-sensitive potassium channels are essential for the regulation of insulin secretion [24]. Sevoflurane may affect insulin secretion through continuous activation of adenosine triphosphate-sensitive potassium channels in β-cells [25]. The direct effects of sevoflurane on insulin secretion underscore the importance of controlling insulin levels, for example, by the hyperinsulinaemic clamp technique, when investigating insulin action in anaesthetized mice.

Skeletal muscle is the major organ for glucose disposal after a glucose load [26], and skeletal muscle insulin resistance is a hallmark of type 2 diabetes [10]. The marked decrease in insulin-stimulated glucose uptake during sevoflurane anaesthesia was most evident in soleus muscle which is characterized by a high content of type 1 fibres [27]. In human beings, abundance of type 1 fibres and whole-body insulin sensitivity are positively correlated [28]. In contrast, type 2 fibres have high glycolytic capacity and are less insulin-sensitive [29]. The pronounced effect on soleus muscle could therefore indicate a direct interaction with insulin-stimulated glucose uptake. In skeletal muscle, insulin stimulates glucose uptake by signalling through PI3k-Akt2 and RabGTPases to facilitate GLUT4 translocation to the plasma membrane [30]. This mechanism is inhibited by high levels of circulating lipids [31], and the observed sevoflurane-associated increase in FFA is likely involved in the impairment of insulin action during anaesthesia. Direct inhibition of glucose uptake in skeletal muscle by sevoflurane cannot be excluded from our data, but previous observations do not support a negative effect on glucose transport. In fact, incubation of L6

rat skeletal muscle cells with sevoflurane increases glucose uptake [32], increases glucose oxidation in isolated working rat hearts [33] and stimulates inositol 1,4,5-trisphosphate production in skeletal muscle [34]. The conscious mice were slightly more physically active than the anaesthetized group. Muscle contractions can stimulate glucose transport independently of insulin stimulation through GLUT4 translocation [35]. This phenomenon is predominantly observed under intense physical activity [36], and using the dual catheter method, we were able to take blood samples from the mice without physical contact, and thereby a minimum level of stress was applied to the animals [15]. It is therefore unlikely that differences in contraction-induced glucose transport alone can explain the lower glucose uptake in skeletal muscle from sevoflurane-treated animals. Additionally, differences in physical activity will not affect glucose uptake through other transporters than GLUT4 as in CNS.

Liver has a central role in regulation of substrate metabolism and glucose homoeostasis [11]. During physiological conditions, EGP is suppressed by insulin, and this effect was preserved during the hyperinsulinaemic isoglycaemic clamp in sevoflurane-anaesthetized mice. Our study was insufficiently powered to exclude differences in insulin suppression of EGP between anaesthetized and conscious mice, but the observed tendency indicated an increased insulin suppression of EGP in sevoflurane-treated animals (p value for interaction = 0.075). This finding has important perspectives for the use of animal models to investigate hepatic substrate metabolism, as it suggests that insulin action in liver is affected to a much lesser degree than in muscle by sevoflurane.

We found a marked decrease in glucose uptake in brain of anaesthetized animals despite higher circulating glucose levels. Glucose transport across the blood–brain barrier and net cerebral glucose metabolism are not affected by insulin in the physiological change or during clamp conditions [37]. It therefore seems unlikely that reduction in glucose uptake in the brain is due to inhibition of insulin action by sevoflurane. The insulin stimulation on brain glucose metabolism is already saturated at fasting concentrations in healthy individuals [37]. The reduction in cerebral glucose uptake could be due to direct effects of sevoflurane on blood flow and/or cerebral blood perfusion. This is supported by observations in human beings where sevoflurane reduces cerebral blood pressure measured by ultrasonography [38]. Ketone bodies provide an alternative to glucose as substrate for oxidation in the brain, and interestingly, sevoflurane has been observed to be associated with increased ketogenesis [39].

In fat tissue, insulin inhibits lipolysis and stimulates glucose uptake. The observed increase in plasma FFA suggests that sevoflurane affects lipid homoeostasis. The underlying mechanism could be impairment of insulin suppression of lipolysis and/or reesterification of FFAs, or reduced lipid oxidation [40]. We did also observe lower glucose uptake, but it did not reach statistical significance (BAT, p value = 0.215, subcutaneous fat, p value = 0.723 epididymal fat, p value = 0.185). These observations could indicate an impairment of insulin action on adipose tissue during sevoflurane administration, but no firm conclusions can be drawn based on the present data. If the sample size had been sufficiently larger to detect a difference in glucose uptake when it exists, we might have minimized the risk of type 2 errors for some of the secondary endpoints like tissue-specific glucose uptake.

Our findings are of importance for the clinical use of halogenated ether group, such as sevoflurane. The combination of impaired insulin action on skeletal muscle and reduced insulin levels can be harmful, especially if the condition is not detected in due time. In patients with diabetes, intravenous propofol anaesthesia prevents increases in blood glucose levels during surgery in comparison with isoflurane anaesthesia [41]. This could suggest that propofol is superior to general anaesthesia in patients with diabetics. Among patients with diabetics, the interaction between sevoflurane and insulin is well recognized, and glucose homoeostasis during anaesthesia can be regulated by infusion of exogenous insulin and glucose. However, severe insulin resistance can be compensated by high endogenous insulin levels like in obese non-diabetic subjects [42] and this condition may not be diagnosed prior to procedures that require the use of anaesthesia. The combined effect of sevoflurane on insulin secretion and skeletal muscle insulin resistance may cause severe dysregulation of glucose homoeostasis during anaesthesia. Consequently, the risk of using sevoflurane in clinical practice is low, due to the fact that most patients maintain normal glucose homoeostasis even during sevoflurane anaesthesia. However, care should be taken when using sevoflurane in patients with known or suspected glucose intolerance or pre-diabetes.

In conclusion, sevoflurane anaesthesia in mice affects whole-body insulin sensitivity, insulin secretion and non-insulin-stimulated glucose disposal. These effects are primarily manifested by suppression of glucose uptake in the brain and peripheral tissues, while hepatic glucose homoeostasis seems to be affected to a lesser extent.

These findings suggest using intravenous anaesthesia, such as propofol, in insulin resistance patients is superior to halogenated ether group of inhalational anaesthetic agents, but this hypothesis remains to be tested in human beings.

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Conflict of Interest

None.

Author Contributions

Kasper Faarkrog Høyer, Thomas S. Nielsen and Jonas T. Treebak designed the study. Kasper Faarkrog Høyer, Thomas S. Nielsen, Steve Risis, Jonas T. Treebak and Niels Jessen analysed the data. Kasper Faarkrog Høyer and Steve Risis performed the research. Kasper Faarkrog Høyer and Niels Jessen wrote the manuscript. All authors read and approved the final version of the manuscript.

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