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Approach to assessing determinants of glucose homeostasis in the conscious mouse

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Abstract Obesity and type 2 diabetes lessen the quality of life of those afflicted and place considerable burden on the healthcare system. Furthermore, the detrimental impact of these pathologies is expected to persist or even worsen. Diabetes is characterized by impaired insulin action and glucose homeostasis. This has led to a rapid increase in the number of mouse models of metabolic disease being used in the basic sciences to assist in facilitating a greater understanding of the metabolic dysregulation associated with obesity and diabetes, the identification of therapeutic targets, and the discovery of effective treatments. This review briefly describes the most frequently utilized models of metabolic disease. A presentation of standard methods and technologies on the horizon for assessing metabolic phenotypes in mice, with particular emphasis on glucose handling and energy balance, is provided. The article also addresses issues related to study design, selection and execution of metabolic tests of glucose metabolism, the presentation of data, and interpretation of results.

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Introduction

The use of mice in biomedical research holds decided importance as evident by the report that in the majority of academic research facilities mice amount to 99 % of the mammalian census (Laughlin et al. 2012). This widespread use is, in part, the result of the mouse genome being well characterized, the availability of numerous mutant mice, and the relatively low husbandry cost and space requirements (Laughlin et al. 2012). Of particular interest is the utilization of mice with the goal of furthering our understanding of diabetes, obesity, and associated complications. To achieve this goal, investigators must consider multiple factors when implementing and conducting mouse metabolic phenotyping tests of these metabolic diseases including those inherent to the mouse model employed, those related to technical procedures, and the presentation of results (Ayala et al. 2010). Factors inherent to the mouse include strain, husbandry, age, and sex. These metabolic determinants have been previously evaluated and discussed in detail by our group (Ayala et al. 2010; Berglund et al. 2008). In this article, we present murine models often utilized in the investigation of metabolic dysregulation and techniques used in the assessment of glucose homeostasis in mice. Recommendations in experiment design, execution, interpretation, and presentation are provided. Also, emerging approaches in the evaluation of glucose metabolism and energy balance are introduced.

Mouse models of metabolic disease

Understanding metabolic diseases is greatly assisted by animal models that mimic the metabolic characteristics of human disease. For example, a mouse model of metabolic

Table 1 Mouse models of metabolic disease

Model	Obesity	Dyslipidemia	Insulin resistance	Fatty liver	Hyper-tension	Cardiac dysfunction	Notes	References
Ob/ob	4 weeks	↓ HDL, 12 weeks	12 weeks	12 weeks	No	20–24 weeks	No leptin signaling, infertility	(Coleman and Hummel 1973; Ingalls et al. 1950; Kennedy et al. 2010; Nishina et al. 1994)
Db/db	4 weeks	12 weeks	12 weeks	20 weeks	No	12 weeks	No leptin signaling, infertility	(Hummel et al. 1966)
A^y/a	8 weeks	↓ HDL, 12 weeks	12 weeks	Yes	Yes	Yes	Tumor formation	(Anstee and Goldin 2006; Dickie 1969; Kennedy et al. 2010; Williams et al. 2003; Yamauchi et al. 2010)
MC4-$R^{-/-}$	10 weeks	ND	Yes	Yes	No	ND	Haploinsufficiency of MC4-R seen in humans	(Huszar et al. 1997; Tallam et al. 2005)
MC3-$R^{-/-}$	12 weeks	ND	No	No	No	No	Increased adiposity w/o increased body weight	(Butler et al. 2000; Chen et al. 2000; Ellacott et al. 2007; Humphreys et al. 2011; Trevaskis et al. 2008)

ND not determined, *HDL* high density lipoprotein

syndrome displaying obesity, dyslipidemia, hypertension, and fatty liver disease is often desired. Here we briefly present mouse models (genetic, pharmacological, and environmental) that are frequently utilized to assess metabolic dysregulation associated with obesity and diabetes (Table 1).

Spontaneous genetic mutant models of obesity and insulin resistance

Genetic mouse models of obesity and diabetes are extremely useful in evaluating specific molecular mechanisms that could be involved in the development of associated pathophysiology in humans. However, diabetes-related metabolic dysregulation in humans is not a monogenic disease. The relevant question is therefore whether these models display metabolic phenotypes that are representative of the human condition. Many of the mouse models commonly utilized in the study of metabolic disease arose from spontaneous mutations. Among these are the ob/ob, db/db, and lethal yellow agouti mice. Although their primary phenotype is obesity, these models display insulin resistance and some degree of dyslipidemia (Coleman and Hummel 1973). Although the mechanisms by which these monogenic mutant mice develop diabetes are decidedly different from the typical diabetic they display similar phenotypes. Thus, these have become valuable mouse models that are heavily used particularly in pharmacology studies.

The leptin-deficient ob/ob mouse

The ob/ob (C57BL/6J-ob/ob) mouse was one of the first genetic models used to study diabetes (Enser 1972). These mice have a monogenetic autosomal recessive mutation in the leptin gene that arose in the Jackson Laboratory (Ingalls et al. 1950). Leptin is an adipokine identified in 1994 (Zhang et al. 1994), which plays a key role in regulating energy intake and expenditure. Leptin-deficient ob/ob mice develop obesity, hyperinsulinemia, and hyperglycemia after 4 weeks of age (Dubuc 1976). The increased body weight compared to the lean littermates is apparent from weaning (Mark et al. 1999). Impairments in glucose tolerance appear after 12 weeks of age (Van den Bergh et al. 2008), alongside hepatic steatosis and inflammation (Bigorgne et al. 2008; Park et al. 2011). This model also develops cardiac fibrosis and decreased cardiac function between 20 and 24 weeks of age (Dobrzyn et al. 2010; Zaman et al. 2004). In contrast to humans with metabolic syndrome, these mice do not develop hypertension or dyslipidemia even after 36 weeks (Mark et al. 1999; Van den Bergh et al. 2008).

The leptin receptor-deficient db/db mouse

The db/db (C57BL/KsJ-db/db) mouse has an autosomal recessive mutation in the leptin receptor gene leading to higher body weights compared to their lean littermates after 6 weeks of age (Dong et al. 2010). The metabolic profiles of the ob/ob and db/db mice are similar. Db/db mice exhibit elevated fasting blood glucose concentrations at 8 weeks of age as well as increased plasma triglycerides, total cholesterol, and non-esterified fatty acids after 13 weeks of age (Ae Park et al. 2006). Hyperinsulinemia and impaired glucose tolerance are observed at 12 weeks of age (Dong et al. 2010; Winzell et al. 2010). These mice develop hepatic steatosis after 20 weeks of age but fail to show hepatic inflammation

and fibrosis (Ge et al. 2010; Sahai et al. 2004). Cardiac inflammation and fibrosis are present at 12 weeks of age, however, blood pressure remains unchanged (Dong et al. 2010). The primary difference between the ob/ob and db/db mice is that db/db mice have dramatic elevations in circulating leptin concentrations, proportional to their increase in adiposity, whereas the ob/ob mice have no circulating leptin (Kennedy et al. 2010).

Agouti lethal yellow (A^y/a) mouse

Several spontaneous mutations leading to placement of the agouti gene under the control of constitutively active promoters result in ectopic ubiquitous expression of the agouti protein (Dickie 1969). In the brain, the agouti protein competes against the anorexigenic factor α-melanocyte-stimulating hormone (MSH) for binding to the melanocortin 4 receptor (MC4-R), acting as an antagonist of this signaling pathway (Yen et al. 1994). As a result, A^y/a mice display adult-onset obesity and insulin resistance due to hyperphagia and hypoactivity (Tschop and Heiman 2001). This is similar to the phenotype displayed by MC4-$R^{-/-}$ mice (see below) (Fan et al. 1997; Huszar et al. 1997; Lu et al. 1994). In addition, the A^y/a mouse is hypertensive (Mark et al. 1999). Particularly useful to investigators is that the obesity phenotype displayed by these mice can be amplified by high-fat feeding. The mice compliment the ob/ob and db/db mice since leptin signaling remains intact.

Genetically engineered mouse models

In recent years, genetically engineered mouse models have been developed to study the role of a particular protein or pathway in the development of diabetes and obesity. Targets of interest have included the melanocortin 3 and 4 receptors, glucose transporter 4 (GLUT4), insulin receptor, and insulin receptor substrate (IRS-1 and IRS-2). These models provide invaluable insight into the role of each protein. However, with the exception of the melanocortin 4 receptor knockout mouse, these site-specific approaches do not completely model the complexity of human metabolic diseases.

The melanocortin 4 receptor knockout (MC4-$R^{-/-}$) mouse

Changes to the homeostatic networks in the central nervous system (CNS) have profound effects not only on weight regulation but also on various other aspects of metabolism. It has been shown that an increase in brain glucose concentration can reduce plasma triglyceride levels (Lam et al. 2007), which may be mediated by the central melanocortin system (Nogueiras et al. 2007). The central melanocortin system is known to mediate many of the actions of leptin and plays a crucial role in the central regulation of energy homeostasis (Cone 2005). Strikingly, alterations in the *MC4R* gene are the most common monogenic cause of obesity known in humans (Hinney et al. 1999; Marti et al. 2003; Mergen et al. 2001; Meyre et al. 2009; Vaisse et al. 1998; Willer et al. 2009; Yeo et al. 1998). Also, mice lacking the melanocortin 4 receptor (MC4-$R^{-/-}$) exhibit many of the phenotypic characteristics of humans with *MC4R* mutations (Farooqi et al. 2003). The MC4-$R^{-/-}$ mouse develops obesity associated with hyperphagia, hyperglycemia and hyperinsulinemia (Huszar et al. 1997). Restricting food intake of the MC4-$R^{-/-}$ mice to the amount consumed by wild types will only partially reduce their weight due to hypometabolism (Ste Marie et al. 2000). These mice are also highly sensitive to high-fat feeding, which will exacerbate their hyperphagia, obesity, and hyperinsulinemia (Sutton et al. 2006). Despite their profound obesity, MC4-$R^{-/-}$ mice tend to be hypotensive (Tallam et al. 2005). This model also develops hepatic steatosis (Sutton et al. 2006) while circulating triglycerides and non-esterified fatty acids appear to remain comparable to wild-type mice (Albarado et al. 2004).

The melanocortin 3 receptor knockout (MC3-$R^{-/-}$) mouse

Alongside the MC4-R, the MC3-R is the other centrally expressed melanocortin receptor that plays a role in regulating energy homeostasis. Lack of MC3-R in mice results in a unique phenotype characterized by an increase in adiposity without increases in body weight, food intake, or impairments in glucose homeostasis (Butler et al. 2000; Chen et al. 2000). Surprisingly, these mice do not develop insulin resistance and hepatic steatosis even when high-fat fed. It has been proposed that this phenotype is, in part, tempered by a reduced inflammatory response to obesity (Ellacott et al. 2007). As such, this model may be useful to study obesity in the absence of metabolic syndrome.

Mouse models lacking specific components involved in insulin signaling

Insulin receptor knockout mice do not survive over 72 h as they develop severe ketoacidosis with hyperglycemia and hyperinsulinemia (Bruning et al. 1998; Jackerott et al. 2001). To circumvent this limitation, mouse models with tissue-specific deletion of the insulin receptor have been developed. Each of these models exhibits a distinct metabolic phenotype which has been extensively reviewed (Nandi et al. 2004). We will provide only a brief overview here of components involved in insulin signaling.

Mice lacking IRS-1 exhibit intrauterine and postnatal growth retardation associated with mild insulin resistance (Araki et al. 1994; Tamemoto et al. 1994). Combined heterozygosity for the insulin receptor and IRS-1 causes severe impairment in insulin action and increased incidence of diabetes in the resulting progeny (Bruning et al. 1997). Mice lacking IRS-2 develop diabetes due to a combination of insulin deficiency due to pancreatic β-cell apoptosis (Kushner et al. 2002) and insulin resistance in peripheral tissues (Kido et al. 2000). Of note, mice lacking both IRS-1 and IRS-2 die before implantation, resulting in one of the most dramatic embryonic lethal phenotypes observed in mice with targeted mutations (Withers et al. 1999).

Insulin-dependent glucose uptake requires GLUT4 translocation from intracellular compartments to the plasma membrane (Leto and Saltiel 2012; Slot et al. 1991). Mice lacking GLUT4 show growth retardation, cardiac hypertrophy, underdeveloped adipose tissue, and moderate insulin resistance and hyperglycemia in the fed state (Katz et al. 1995). Interestingly, heterozygous deletion of GLUT4 induces a more severe phenotype of insulin-resistant diabetes without obesity (Stenbit et al. 1997).

Although mice deficient for proteins of the insulin signaling pathway are invaluable in the study of insulin action, they are limited in the study of diabetes as they develop few of the symptoms of this metabolic disease. Also, they often exhibit numerous other confounding issues such as growth retardation.

Chemically induced models of diabetes

Alloxan and streptozotocin are structural analogs of glucose that enter pancreatic β-cells via the GLUT2 transporter (Lenzen 2008). Single injections of either of these molecules induce selective necrosis of β-cells in rodents as a model of type 1 diabetes (Lenzen 2008). Chemically induced diabetic mice usually exhibit undetectable levels of insulin and hyperglycemia but do not show the diverse characteristics of the metabolic syndrome (Jourdan et al. 2009). Type 2 diabetes can be induced by multiple exposures of low-dose streptozotocin (Paik et al. 1980) or, more successfully, by a combination of low-dose streptozotocin and a high-energy diet (Srinivasan et al. 2005). This combination induces hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and cardiac dysfunction. Streptozotocin has well-described adverse side effects, such as hepatotoxicity and nephrotoxicity (Palm et al. 2004; Schein and Loftus 1968). In addition, the key signs of hypertension and obesity have not been reported.

Recently, *N*-ethyl-*N*-nitrosourea (ENU) mouse mutagenesis projects have been conducted using hyperglycemia as a screening parameter (for review see (Aigner et al. 2008)). ENU–induced mutagenesis allows for the production of a large number of randomly mutant offspring from treated males. This method will predominantly induce point mutations, leading to null, partial loss of function or gain of function alleles. Mouse lines can then be established according to a phenotypic criteria, such as hyperglycemia, thereby revealing a mutation as cause for the phenotype. ENU mutations have led to the identification of novel alleles in genes already known to be involved in glucose homeostasis including glucokinase (Inoue et al. 2004; van Burck et al. 2010) and leptin (Hong et al. 2010; Osborn et al. 2010) as well as genes previously unrelated to glucose metabolism, such as the gene encoding the histone methyltransferase *Mll2* (Goldsworthy et al. 2013). ENU-induced mutagenesis is a strong method in that it can generate a wide array of subtle phenotypes and the resulting lines may more closely recapitulate the human phenotype compared to engineered knockout models. Diet challenges are now being employed in this model to identify genetic and environmental interactions in the pathogenesis of metabolic disease (Lee et al. 2012; Svenson et al. 2008).

Diet-induced metabolic syndrome

Diet composition plays an extremely important role in the development of metabolic dysregulation. The modern diet, particularly in western countries, is rich in carbohydrates such as fructose and sucrose as well as saturated fat. This high-energy intake has been associated with metabolic syndrome, cardiovascular disease, and nonalcoholic fatty liver disease (Lim et al. 2010; Massiera et al. 2010). Therefore, combinations of high-fat and high-carbohydrate diets have been extensively used in rodents to precipitate phenotypes that are closely related to the human metabolic syndrome. It is important to note that different genetic strains of mice will have very different responses to a high-calorie diet. The C57BL/6 J, AKR/J, and DBA/2 J mice have been reported to be more prone to the development of obesity and insulin resistance than the SWR/J, A/J, and 129S6 lines (Alexander et al. 2006; Surwit et al. 1988).

High-fat diets for diet-induced obesity

Mice will readily gain weight when provided a high-fat diet, but the level and source of fat will influence the severity of the resulting phenotype and should be considered in the experimental design. It is recommended that chow or low-fat diets (LFD), used as control diets, have 10 % calories from fat. In contrast, high-fat diets (HFD) have 30–50 % calories from fat, and very high-fat diets (VHFD) contain over 50 % calories from fat. There is a dose response for body weight as a function of dietary fat,

however, both HFD and VHFD induce obesity. In addition, variable responses are also observed in glucose tolerance, insulin resistance, and triglyceride levels depending on the source of dietary fat (Ikemoto et al. 1996; Wang et al. 2002). High-fat feeding for over 4 weeks with both animal and plant fat-enriched diets leads to obesity, hyperglycemia, hyperinsulinemia, impaired glucose tolerance, hepatic steatosis, increased circulating triglycerides and non-esterified fatty acids (Buettner et al. 2007), and impaired cardiac function (Kobayasi et al. 2010). However, the onset of metabolic perturbations has been shown to occur more rapidly when animal fat is used in HFD (Buettner et al. 2007).

High fructose/sucrose diets for hypertriglyceridemia and insulin resistance

Increased intake of fructose or the disaccharide sucrose (composed of fructose + glucose) is associated with obesity, hypertriglyceridemia, and insulin resistance in humans (Basciano et al. 2005). In rodent models, diets containing high fructose or sucrose induce many characteristics associated metabolic syndrome including dyslipidemia, insulin resistance, and fatty liver (Basciano et al. 2005; Tran et al. 2009). Typically, control diets contain about 4 % of sucrose and <0.5 % of free fructose, but formulas of control diets should be chosen wisely as levels of sucrose may vary. A high fructose/sucrose diet will contain 50–60 % calories from fructose or sucrose. It appears the fructose component of sucrose is largely responsible for the deleterious effects of the high-sucrose diet. Fructose, unlike glucose, does not induce insulin secretion due to the absence of the fructose transporter on the pancreatic β-cell (Bray et al. 2004). Also, fructose does not stimulate leptin secretion (Bray et al. 2004), however, it facilitates de novo lipogenesis in the liver (Basciano et al. 2005).

The established model of sucrose-induced insulin resistance and hypertriglyceridemia is the rat, which develops a phenotype within 2 weeks of initiating the diet. Rats will also exhibit high blood pressure and impaired cardiac function (Tran et al. 2009). Unless fed for a prolonged period of time, this diet does not induce substantial weight gain (Chicco et al. 2003). In contrast to rats, the mouse is less frequently used as a model of sucrose-induced insulin resistance as its response to high fructose/sucrose diet is highly strain dependent. Commonly used strains such as C57BL/6 mice do not develop insulin resistance in response to high-sucrose diets (Nagata et al. 2004; Sumiyoshi et al. 2006).

Choosing a mouse model of metabolic disease

In mice, there are numerous naturally occurring and gene-targeted mutations as well as diet interventions that promote obesity and metabolic dysregulation associated with the human metabolic pathophysiology. Care should be taken when choosing an animal model for metabolic studies, taking into account the diet used as well as the degree to which they develop obesity, hyperglycemia, hyperlipidemia, insulin resistance, and hypertension. There is, of course, no perfect animal model of the human disease. Each model described has specific attributes that make them useful for studying the metabolic phenotypes of obesity, diabetes, and metabolic syndrome. The specific mouse model depends on the experimental design and hypothesis of the study. For reviews with a more in-depth description of models, see (Aigner et al. 2008; Buettner et al. 2007; Guo 2014; Kennedy et al. 2010; Nandi et al. 2004; Panchal and Brown 2011).

Diagnostic tests for metabolic disease

With the proliferation of mouse models of metabolic diseases has come a great need to develop tests to characterize them. The most common tests applied to the mouse are those that have been taken from the clinic and human literature. There are obvious obstacles in studying the mouse. They are small, easily stressed by restraint and handling, and they have a small blood volume. This section summarizes the tests that are often used and how they are interpreted. Additional assessment of these tests can be found elsewhere (Ayala et al. 2010; McGuinness et al. 2009; Wasserman et al. 2009).

The primary screen for glucose intolerance and insulin sensitivity is measurements of blood glucose and insulin. As with patients, blood hemoglobin A1c is a valid index of long-term glycemic state in the mouse (Han et al. 2008). This could be of value in characterizing mice since hemoglobin A1c concentrations are independent of the stress of mouse handling and blood drawing in the absence of an implanted catheter (Han et al. 2008).

A continuous thread that complicates more advanced screens is that interpretation of these tests is dependent on the dosing (McGuinness et al. 2009) and expression of results (Kaiyala et al. 2010; Kaiyala and Schwartz 2011) in mice of different weights and body composition. Accurate measurement of body weight and composition is imperative for optimal interpretation of metabolic test results. Strict adherence to ensuring these measurements are obtained from the same equipment at a similar time of day will assist in minimizing erroneous conclusions. Also, more advanced modeling of the impact of different tissue types on dosing and metabolic readouts will be a fruitful future direction.

Despite widespread variation between laboratories in how metabolic phenotyping tests are conducted and the

difficulty of such tests, technical descriptions are usually poor (Wasserman et al. 2009). Doses, sampling sites, time courses, and insulin measurements are frequently omitted. As such, McGuinness and colleagues have produced a list of best reporting practices (McGuinness et al. 2009).

Glucose tolerance tests

A glucose tolerance test (GTT) is exactly what the name purports it is. It is not a reflection of insulin sensitivity or insulin secretion, in and of itself. It is simply a measure of the body's ability to dissipate changes in blood glucose in response to a glucose load. The glycemic excursion is determined by multiple factors that require other tests to distinguish. The most common ways to administer glucose in the mouse are via gavage or intraperitoneal injection and samples are obtained through stressful approaches that require handling and acute venipuncture. Non-stressful techniques have been developed to deliver the glucose load by indwelling catheters into the stomach (Bonner et al. 2013) or the vein (Alonso et al. 2012; Morton et al. 2013) while sampling from an indwelling catheter in the artery. Catheters are implanted under general anesthesia a week prior to testing. The advantage of implanting a catheter in advance is that mice are not handled, restrained, or stressed during the experiment.

Glucose tolerance is a valuable clinical tool that has now been broadly applied to the mouse. Several differences exist between how glucose tolerance tests are implemented in humans versus mice. First, in humans, the glucose load is given orally in a clinical setting. With the notable exceptions described in the preceding paragraph, glucose in mice is administered into the peritoneum or by gavage. The second major difference is that with a GTT in humans a fixed dose of glucose is given independent of patient weight. The typical approach in mice is to base the dose of glucose on the weight of the mouse, usually at 1–2 g/kg. This is reasonable as long as the weight and body composition for different cohorts are similar. However, the increased weight, which is common in mouse models of metabolic disease, is typically due to a higher fat mass. This is an important consideration, as lean mass is the principal site of glucose disposal. If a glucose dose is administered based on total body weight, then the dose given to an obese mouse will be greater than the non-obese control. The consequence is that the obese mouse may appear glucose intolerant by virtue of the higher glucose dose and not due to an actual metabolic difference. If mouse body composition is known then it is more appropriate to base the dose of glucose on lean body mass (McGuinness et al. 2009).

The standard presentation of results from a GTT is a description of blood glucose over time after the glucose dose. A time course of blood glucose is valid as long as the groups being compared have equivalent fasting glucose levels. When fasting glucose levels differ, as is often the case with diabetic or insulin-resistant models, a calculation of the area under the curve facilitates interpretation. Interpretation of a GTT can also benefit greatly from presentation of a time course of insulin levels.

While much variation in the sampling frequency followed during a GTT in the mouse exists, our laboratory has previously measured arterial glucose at 5, 10, 15, 20, 30 45, 60, 90, and 120 min after glucose administration (Bonner et al. 2013). Arterial insulin concentrations during the GTT were evaluated at 10, 20, 30, 60, and 120 min (Bonner et al. 2013). This time course offers a compromise between obtaining samples for appropriate evaluation of glucose excursion and avoiding a fall in hematocrit resulting from excessive sampling. Recently, the frequently sampled intravenous glucose tolerance test (FSIVGTT) originally developed by Bergman and colleagues in humans (Bergman et al. 1981) has been adapted for mice (Alonso et al. 2012; Morton et al. 2013). The requirement for frequent sampling of glucose and insulin in the FSIVGTT necessitates an indwelling sampling catheter preferably in the artery. This test, although assumption dependent, is very powerful in that it provides a read out of insulin sensitivity, glucose effectiveness, insulin secretion, and the glucose disposition index in one test.

Insulin tolerance test

As with the GTT, the insulin tolerance test (ITT) relies on the measurement of glucose concentration over time but these measures are in response to an insulin bolus rather than a glucose bolus. In the mouse, the insulin bolus is given as an intraperitoneal or intravenous injection. The ITT has been used in humans for many years to test hypothalamo-pituitary-adrenal axis response (ITT_{HPA}) to hypoglycemia (Plumpton and Besser 1969). The ITT was subsequently adapted to measure insulin sensitivity (ITT_{IS}) in humans (Akinmokun et al. 1992). The ITT_{HPA} and the ITT_{IS} that have been applied to humans are much different protocols. The ITT_{HPA} is 90–120 min and samples are taken infrequently for insulin counterregulatory hormones. The ITT_{IS} is a short test ($\sim$15 min) where samples are taken every minute for glucose and the first-order rate constant for the fall in glucose is calculated as a measure of insulin sensitivity (Akinmokun et al. 1992). In a previous commentary, it was highlighted how tests originally developed in humans have been lost in translation to the mouse (Wasserman et al. 2009). The ITT is a profound example. To be brief, scientists working in mice attempt to measure insulin sensitivity almost exclusively using the ITT_{HPA}. The longer ITT_{HPA} that is used in mice may

provide valuable information. However, its relationship to insulin sensitivity is untested. The half-life of insulin is ~10 min in mice (Cresto et al. 1977). Therefore, differences in the glucose concentration after the initial fall might not reflect insulin action. Also, when a mouse's blood glucose levels fall below ~80 mg/dl the counter-regulatory response to insulin is activated (Jacobson et al. 2006a, 2006b).

Some of the issues regarding how GTTs should be performed also apply to ITTs. The dosage of insulin administered is often based on body weight, which may be inappropriate in certain instances (McGuinness et al. 2009). An obese mouse (i.e., a mouse with increased fat mass) will receive a larger dose of insulin than a lean mouse even though the mass of insulin-sensitive tissue (lean mass) might not differ proportionately to the difference in total body mass. Thus, normalizing the insulin dose to lean mass, if such information is available, is a more accurate means of determining the insulin dose. A common method for presenting blood glucose from an ITT is as a percentage of basal glucose. This is valid if the groups being compared have similar fasting glucose levels. However, if fasting glucose differs among groups, interpretation of a relative fall in glucose can lead to erroneous conclusions.

Pyruvate tolerance test

The pyruvate tolerance test (PTT) is the glycemic excursion in response to an intraperitoneal or intravenous injection of pyruvate. It is subjected to all the potential complications as the ITT and GTT due to differences in body weight or composition. The classic interpretation of this test is that the change in glucose in response to a bolus of pyruvate is a reflection of hepatic gluconeogenesis. This is an oversimplification as pyruvate is utilized by many tissues and can possibly affect glucose concentrations by competition for oxidative processes in extrahepatic tissues. The advantage of the PTT, as with the ITT and GTT, is simplicity. The PTT is of little value alone, but may be of use if there are substantive other data that may be more directly link data to effects on gluconeogenesis.

Glucose clamping the conscious mouse

In contrast to the GTT, ITT, and PTT where the variable of interest is the blood glucose excursion, the goal of the glucose clamp is to fix blood glucose at a precise, well-controlled concentration. There are three types of glucose clamps that have been performed. These are the hyperinsulinemic, euglycemic clamp, the hyperinsulinemic, hypoglycemic clamp, and the hyperglycemic clamp. The first tests insulin action, the second tests the response to insulin-induced hypoglycemia, and the third tests insulin secretion. The difficulty in standardizing the clamp has been due to the lack of standardization in reporting (Ayala et al. 2006; McGuinness et al. 2009; Wasserman et al. 2009). Consequently, there is considerable variation in clamp protocols and a great deal of mystery regarding standard features of how the clamp was performed (e.g., how blood is obtained, isotope delivery, frequency of sampling, etc.). This mystery is all the more confounding as the glucose clamp, done properly, is a very difficult technique. It would seem that the majority of clamps are performed in restrained, conscious mice, where blood is obtained after the tail is cut (Fig. 1a). Although stress is never measured in this preparation, one might predict a restrained mouse with blood being squeezed from its cut tail is stressed. Infusions of insulin, glucose, and isotopes are given via an indwelling jugular vein catheter. The Vanderbilt Mouse Metabolic Phenotyping Center (MMPC) has made efforts

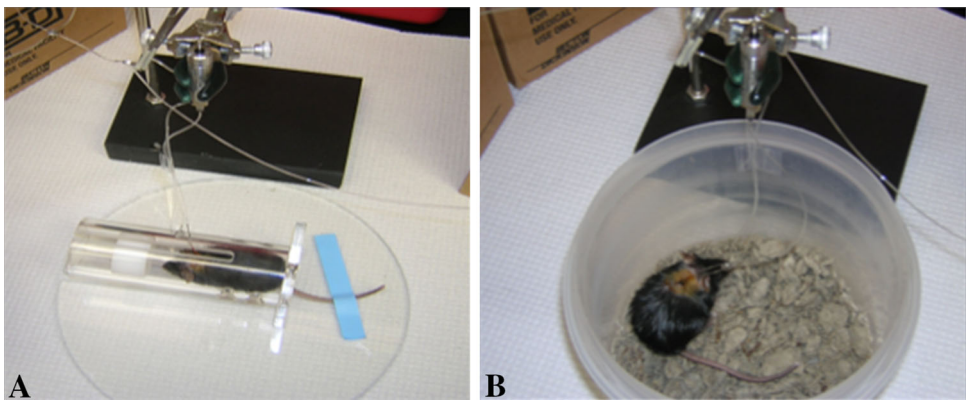

Fig. 1 Depiction of variations in acquiring blood samples during glucose clamps in conscious mice. **a** Blood samples may be obtained via a tail cut. Sampling from the cut tail necessitates restraint of the mouse and the tail, and handling of the tail throughout the duration of the experiment. **b** Blood sampling via a chronic, indwelling, arterial catheter. Sampling from an arterial catheter does not require restraint and handling throughout the experiment

over the last 15 years to improve on this and maintains a downloadable protocol online (https://www.mc.vanderbilt.edu/root/vumc.php?site=mmpc). The Vanderbilt MMPC protocol is unique in that catheters for arterial sampling and venous infusion are implanted a week prior to the experiment. During the experiment, mice are not restrained or handled, and remain conscious and free-roaming (Fig. 1b). Measurement of arterial catecholamines shows that this mouse model is stress free.

Hyperinsulinemic, euglycemic clamp

The hyperinsulinemic, euglycemic clamp, or insulin clamp, is widely considered the gold standard for assessing insulin action in vivo. The miniaturization of the insulin clamp for use in mice has greatly advanced research in the area of metabolic disease. Several different permutations of this method are currently being used in mice. However, some of these variations are neither intuitive nor well described. One might attempt to compare or interpret results from different laboratories when meaningful comparisons or interpretations are impossible. The general principle of an insulin clamp and experimental set-up is straightforward (Fig. 2a, c). Prior to the clamp, mice are fasted to prevent the appearance of glucose absorbed by the gut. The mice transition to a fasted state quite rapidly. An overnight-fasted mouse is nearly glycogen depleted and will be more insulin sensitive than a short-term fasted mouse (Ayala et al. 2006, 2010). Following the fast, mice receive a constant infusion of insulin to achieve a steady-state hyperinsulinemia above fasting insulin levels, but ideally within the physiological range. The constant infusion can be preceded by a priming dose to more quickly achieve steady-state insulin levels. The increased insulin suppresses endogenous glucose production and stimulates glucose uptake. A variable glucose infusion is administered to maintain euglycemia. The glucose infusion rate is determined by measuring blood glucose at regular intervals. Because of the high fractional blood glucose turnover of the mouse, a minimum glucose-sampling rate of every 10 min is recommended, and the glucose infusion rate is adjusted accordingly. This variable rate of glucose infusion indicates whole-body insulin action, as mice with enhanced insulin action require a greater infusion of glucose. Isotopic tracer infusions can be applied to the general clamp protocol to assess sites where insulin action is affected. Please see "Use of Isotopic Glucose Tracers" for a more detailed discussion of the use of isotopic tracers in the evaluation of glucose flux for the conscious mouse. Hematocrit during a typical insulin clamp will fall to 30 % or less if blood cells are not replaced. Infusion of saline-washed erythrocytes from a strain-matched donor mouse prevents a fall in hematocrit caused by blood sampling.

Hyperinsulinemic, hypoglycemic clamp

The insulin-induced hypoglycemic clamp is used to test the neuroendocrine response to hypoglycemia (Berglund et al. 2008; Jacobson et al. 2006a, 2006b). The protocol is much like that used for the hyperinsulinemic, euglycemic clamp except that the insulin infusion is used to create a supra-physiological insulin concentration (Fig. 2a, c). Also, the rate of glucose fall and plateau glucose levels is carefully controlled to match glucose concentrations between groups. The high insulin concentrations will lead to a precipitous fall in blood glucose. The glucose infusion rate required to perform a hypoglycemic clamp is an index of the integrated effectiveness of the counterregulatory response to low blood glucose. The less glucose that is required the more effective is the counterregulatory response. This protocol is very difficult to do with cut-tail sampling. Arterial catheter sampling is necessary to obtain rapid glucose samples at predictable times to match the fall in blood glucose. In addition, sampling for insulin counterregulatory hormones requires a larger blood volume, which is difficult to obtain from the cut tail of the mouse but, again, is more reasonable when sampling from an arterial catheter.

Hyperglycemic clamp

Hyperglycemic clamps (Fig. 2b, c) are used to assess the pancreatic response to hyperglycemia (Berglund et al. 2008; Nunemaker et al. 2006, 2005). Following a short-term fast, blood samples are obtained for measurement of baseline glucose, insulin, and C-peptide. The clamp begins with a glucose priming dose to quickly achieve target hyperglycemia, typically $\sim$100 to 150 mg/dl above fasting glucose values. The priming dose, which can vary depending on the mouse model, is calculated based on the target hyperglycemia and the volume of distribution of glucose. Glucose is then infused at a variable rate to maintain hyperglycemia for the duration of the experiment, which is typically 2 h. As in other clamp protocols, a fall in hematocrit resulting from repeated blood sampling can be prevented by infusing saline-washed erythrocytes. Performing a hyperglycemic clamp using cut-tail sampling can be difficult because of the frequency of sampling and the volume of blood required for insulin and C-peptide analysis. As previously discussed, massaging the tail to obtain larger blood volumes is stressful to the mouse. Therefore, sampling via an arterial catheter is preferred. A time course for insulin, c-peptide, and glucose is very important so that effects of hyperglycemia on first-phase and second-phase insulin secretion can be ascertained (Nunemaker et al. 2006). An added dimension to the study of insulin secretion in vivo using the hyperglycemic clamps is by isolating islets from the same mice after the experiment (Nunemaker

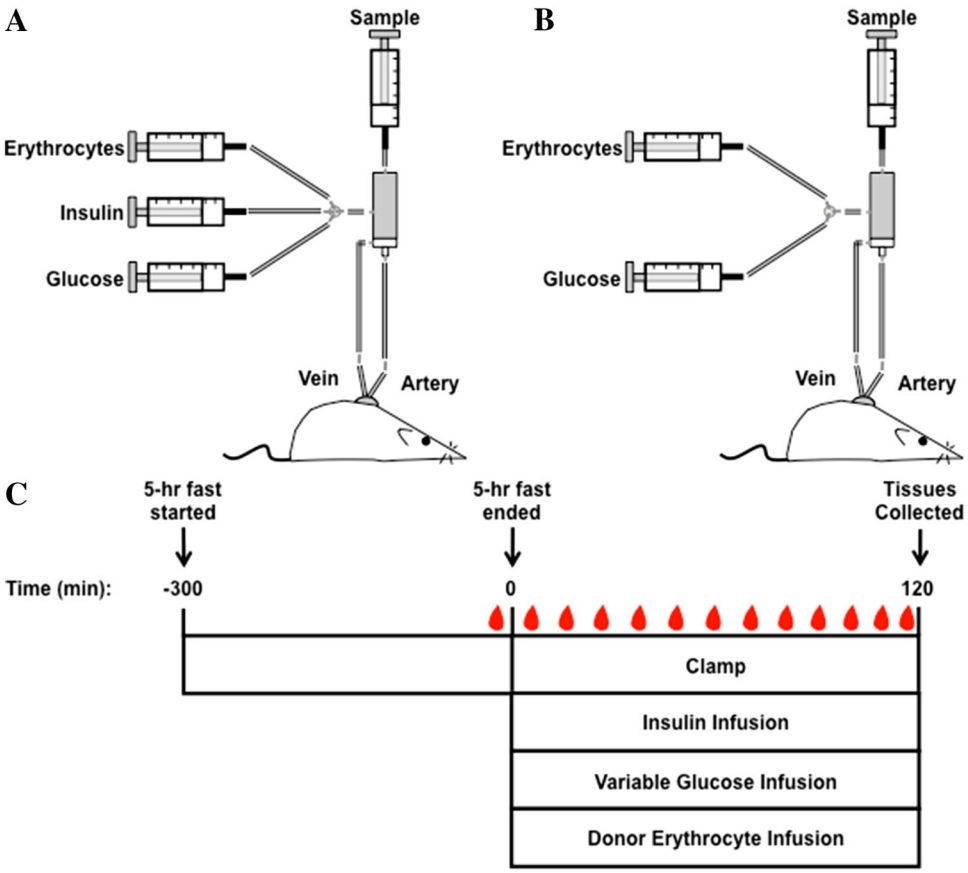

Fig. 2 Experimental set-up and timeline of glucose clamps in the conscious mouse. **a** To complete hyperinsulinemic, euglycemic clamps and hyperinsulinemic, hypoglycemic clamps the Vanderbilt Mouse Metabolic Phenotyping Center (MMPC) connects mice to a dual-channel swivel that allows venous infusion and arterial sampling protocols. This allows for venous administration of insulin, glucose, and erythrocytes. Arterial blood samples to measure glucose and insulin concentrations are obtained from the carotid artery. **b** Vanderbilt (MMPC) set-up of the hyperglycemic clamp to evaluate insulin secretory function. Venous catheterization allows for infusion of glucose and erythrocytes. An arterial catheter provides samples for measurement of blood glucose, insulin and c-peptide levels. **c** A schematic of the timeline of the glucose clamp experiments

et al. 2006). Islet insulin secretion can then be studied using static incubation or perfusion of the islets. Nunamaker et al. (2006) showed that the islets maintain their in vivo oscillatory frequency even after isolation.

Use of isotopic glucose tracers

The glucose clamps described in the *Diagnostic Tests for Metabolic Disease* section are sophisticated techniques that provide the experimenter a means of evaluating in vivo glucose homeostasis at the systemic level. Glucose tracers (radioactive and stable isotopes) may be used in combination with glucose clamps to provide a more directed characterization of glucose kinetics such as identifying endogenous glucose production and tissue-specific glucose flux.

Radioactive isotope methods in the assessment of glucose metabolism

^{3}H-glucose radioisotopes are commonly used to assess whole-body glucose turnover. For example, [3-^{3}H]glucose is metabolized resulting in the loss of the ^{3}H to H2O when it passes through the triose phosphate isomerase step of glycolysis (Vella and Rizza 2009). The measurement of arterial glucose turnover is strongest in the steady state, since the measurement is not dependent on assumptions relating to body glucose space. Steady-state glucose can be ensured with a rigorous glucose clamp. At the Vanderbilt MMPC, we perform the clamp with a mixture of labeled and unlabeled glucose. This approach is effective at maintaining glucose-specific activity at a steady state. This is achieved experimentally (Fig. 3) by a primed-continuous infusion of the [3-^{3}H]glucose tracer initiated at the onset of the equilibration portion of the experiment (Ayala et al. 2011). The primed administration allows for acquisition of a steady-state tracer pool more rapidly during the constant [3-^{3}H]glucose infusion (Ayala et al. 2011). If performing a hyperinsulinemic variation of the glucose clamp some laboratories conduct a second larger [3-^{3}H]glucose prime at the end of equilibration period just prior to the insulin administration (Camporez et al. 2013; Preitner et al. 2009). This is a conceptual error that unfortunately was performed in countless papers in the mouse literature. The repeated [3-^{3}H]glucose prime disrupts the glucose-specific activity equilibrium, promotes a large influx of glucose tracer, and results in artificially elevated appearance of $^{3}H_2O$ and labeling of glycogen stores (Ayala et al. 2010). We suggest that the experimenter mix [3-^{3}H]glucose with the exogenous glucose supply used to clamp glucose. Therefore,

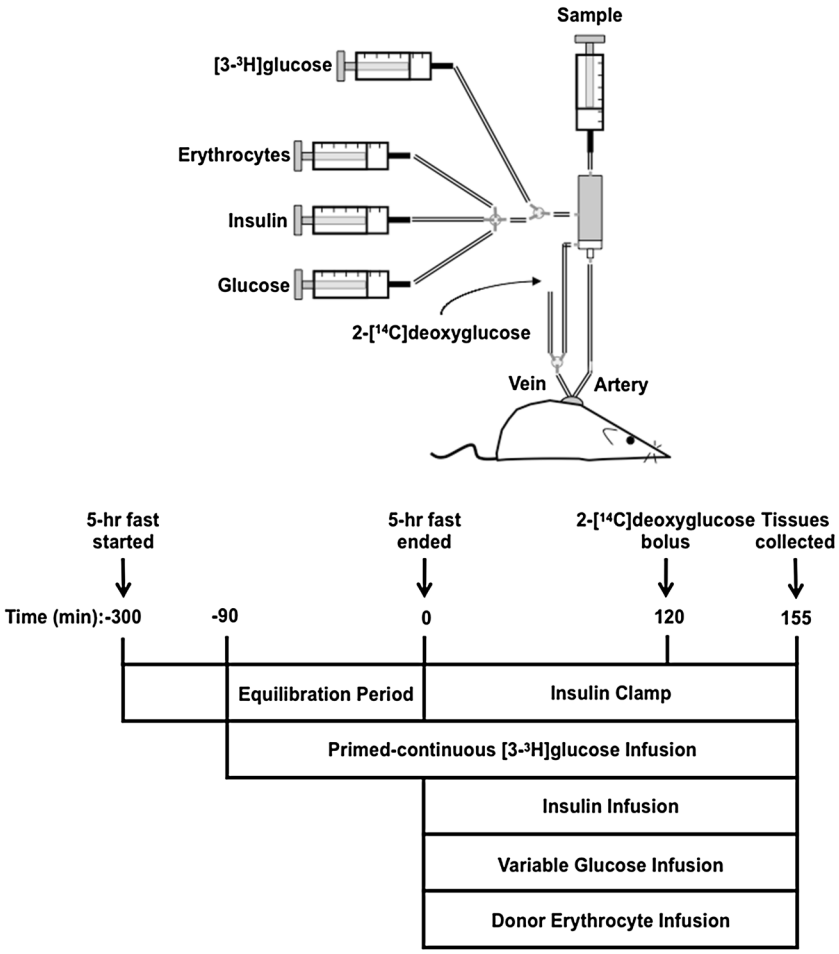

Fig. 3 Experimental schematic for hyperinsulinemic, euglycemic clamp in mice combined with isotopic tracer administration. Mice are fasted for 5 h prior to insulin infusion. A primed-continuous infusion of [3-^{3}H]glucose is infused starting 90 min before to the insulin clamp period. Blood samples are acquired at the conclusion of the equilibration period for evaluation of parameters including arterial glucose, basal glucose turnover, and insulin concentration. The insulin clamp period consists of a constant insulin infusion and a variable glucose infusion. The variable glucose infusion is altered accordingly to maintain euglycemia. Saline-washed erythrocytes are provided to prevent a fall in hematocrit resulting from sampling. From $t = 80$–120 min, arterial blood samples are obtained to assess glucose turnover under steady-state conditions. At 120 min a bolus of 2-[^{14}C]deoxyglucose is administered to provide an index of tissue-specific glucose uptake

every time exogenous glucose is altered the isotopic glucose is altered proportionately and steady state is maintained.

Isotopic 2-deoxyglucose (^{3}H and ^{14}C) has been utilized to provide the metabolic index of glucose, an estimate of tissue-specific glucose uptake, for a variety of rodent tissues including muscle and brain (James et al. 1985; Kraegen et al. 1985; Sokoloff et al. 1977). 2-deoxyglucose administration is an effective means of estimating tissue glucose uptake as it is transported into the cell and phosphorylated in a similar manner to glucose without being metabolized further (Sokoloff et al. 1977). Previous reports have indicated that insulin-mediated glucose uptake differs between muscle types (Ayala et al. 2010; James et al. 1985; Kraegen et al. 1985). It is therefore highly relevant to report the specific muscles studied.

Stable isotopes in the analysis of glucose kinetics

Radioisotopes provide a method for evaluating rates of endogenous glucose production, however, they do not readily allow for the assessment of gluconeogenic and glycogenolytic metabolite flux through contributing metabolic networks. Use of stable isotopes in metabolic studies has experienced a resurgence as the integrated utilization of ^{2}H/^{13}C stable isotopes and nuclear magnetic resonance (NMR)-based isotopomer analysis has facilitated the ability to evaluate nutrient flux from the multiple metabolic pathways based on the positional isotope enrichment produced on glucose. $^{2}H_2O$ administration to enrich body water has provided a measure of the relative contributions of glycogen, glycerol, and phosphoenolpyruvate to endogenous glucose production by measuring ^{2}H-labeling of carbon-bound hydrogens of plasma glucose (Jin et al. 2013; Jin et al. 2004; Jones et al. 2001; Sunny et al. 2011). ^{13}C-glycerol is also currently used to identify the contributions of glycerol to glucose fluxes (Oosterveer et al. 2012). However, work by Previs et al. indicates that use of glycerol isotopomers does not always achieve constant enrichment of glyceraldehyde 3-phosphate (Previs et al. 1995). As such, the reliability of stable isotopes of glycerol for measurement of gluconeogenesis and glucose production may be problematic in some instances. [U-^{13}C]propionate infusion combined with the NMR analysis of plasma

glucose carbon 2 enrichment allowed assessment of phosphoenolpyruvate, pyruvate cycling, and cataplerosis relative to TCA cycle flux (Jin et al. 2013; Jin et al. 2004; Jones et al. 1997; Jones et al. 2001; Sunny et al. 2011). Finally, ^{13}C-glucose administration has granted the ability to extend beyond proportional contributions to endogenous glucose production and determine absolute rates (Jin et al. 2013; Jin et al. 2004; Jones et al. 2001; Sunny et al. 2011).

Unfortunately, the large volumes ($\sim$0.75 ml) of plasma required for the NMR approach have limited its use in the mouse in vivo (Burgess et al. 2005; Satapati et al. 2012). Recently, a gas chromatography-mass spectrometry (GC–MS) approach has been employed to determine the glucose ^{2}H enrichment from three distinct glucose derivatives that result in six independent GC–MS fragment ions using approximately 40 µl of plasma (Antoniewicz et al. 2011). It should be noted that MS cannot provide position-specific enrichment. Given this, a computational isotopomer modeling analysis is then used to interpret the GC–MS enrichment data and calculate metabolic fluxes. Future studies should identify whether the combination of ^{13}C and ^{2}H isotopes, and greater sensitivity of the GC–MS approach can provide comparable fluxes to that of the NMR technology in vivo to mitigate the need for large sample volumes and allow nutrient flux measurements to be scaled down for routine use in murine phenotyping.

Combining metabolomics and metabolic flux analysis in murine phenotyping

Whether a targeted analysis or non-targeted profiling approach is performed, metabolomics provides comprehensive assessment of metabolites and small molecules in a biological system (Castle et al. 2006). Although these technologies allow a greater understanding of metabolic alterations under various physiological conditions and pathologies, the measures obtained from metabolomics are static and require caution when making definitive mechanistic conclusions concerning the functionality of integrated metabolic networks. The application of NMR-based isotopomer analysis of metabolism in combination with MS-driven stationary measures of metabolites can bring much more clarity to metabolic pathways following induction of experimental conditions (Bain et al. 2009). Recently, partnering these two technologies has highlighted that changes in TCA cycle flux following diet-induced hepatic insulin resistance may not always match those in β-oxidation in C57BL/6 mice (Satapati et al. 2012). Thirty-two weeks of high-fat feeding promoted a depression in fasting ketogenesis, and genes involved in hepatic fatty acid transport into the mitochondria and β-oxidation were lowered (Satapati et al. 2012). In contrast, TCA cycle flux was elevated in the high-fat-fed mice (Satapati et al. 2012). This seemingly paradoxical characterization was provided some resolution by performing targeted MS analysis of acylcarnitines in the liver. The short-chain acylcarnitine (C2, C3, C4, and C5) species were elevated in the animals consuming the high-fat diet (Satapati et al. 2012). Through the combination of metabolomics and isotopomer flux analysis, the authors were able to conclude that despite potential for impaired β-oxidation, TCA cycle flux was not likely at risk for being limited by acetyl-CoA provision.

Provoking metabolic phenotypes that are silent in the basal, fasted state

Integrated, in vivo metabolic networks and compensatory pathways prevent overt deviations in glucose homeostasis (Wasserman 2009). Given this, alterations in glycemic control following experimental manipulation may not be easily identified. The clinical assessment of suspected coronary artery disease is a readily apparent illustration of how stressors can be used to uncover phenotypes that may be masked otherwise (Sharma et al. 2012). Exercise stress testing, with emphasis on identifying a depression in the isoelectric period (ST segment) following the QRS complex of an electrocardiogram, is a commonly employed clinical diagnostic tool used to evaluate the presence of cardiac dysfunction (Sharma et al. 2012). Similar to the clinical setting, variations in physical activity level are invaluable in uncovering alterations in metabolism resulting from genetic variation in murine models. For example, Lee-Young et al. (Lee-Young et al. 2010) aimed to determine the role of endothelial nitric oxide synthase (eNOS) on whole-body and tissue-specific glucose metabolism. Under sedentary conditions, wild-type and $eNOS^{-/-}$ mice displayed comparable arterial glucose levels and skeletal muscle glucose utilization (Lee-Young et al. 2010). In contrast, $eNOS^{-/-}$ mice exhibited enhanced skeletal muscle glucose uptake and a resultant hypoglycemia when undergoing an acute bout of treadmill running. Thus, when challenged with exercise an observable phenotype was evoked by the absence of eNOS (Lee-Young et al. 2010).

Provocative stimuli employed to reveal ‘hidden’ physiological characteristics are not limited to exercise-related interventions. Cold exposure (Hirschey et al. 2010), aging (Goren et al. 2004), long-term fasting (Burgess et al. 2005), pharmacological agents (Hasenour et al. 2014), and dietary modification (Kang et al. 2011, 2014) are sensitizers frequently used to evaluate metabolic regulation. Our laboratory and the Vanderbilt MMPC often employ dietary manipulation as such a phenotyping tool. Recently, the use of high-fat feeding in mice has revealed the importance of extracellular matrix (ECM) components in the promotion

of insulin resistance (Kang et al. 2011, 2014). Kang et al. (2011) utilized hyperinsulinemic, euglycemic clamps coupled with isotopic tracers to evaluate systemic and tissue-specific, insulin-stimulated glucose uptake in the absence of integrin $\alpha_2\beta_1$. Integrin $\alpha_2\beta_1$ is a cell surface receptor that interacts with ECM and mediates extracellular/intracellular signaling (Hynes 2002). The lack of this integrin isoform did not have an effect on glucose homeostasis in lean animals (Kang et al. 2011). Following 20 weeks of high-fat feeding, integrin $\alpha_2\beta_1$-deficient mice exhibited improved insulin sensitivity and skeletal muscle glucose uptake despite ECM expansion (Kang et al. 2011). In a follow-up study, insulin action was evaluated in chow and high-fat fed mice lacking matrix metalloproteinase (MMP-9). MMP-9 is a proteinase that promotes the degradation and removal of ECM components from tissue (Nagase et al. 2006). Glucose metabolism was not significantly altered in the chow-fed mice harboring the MMP-9 deletion (Kang et al. 2014). However, animals consuming 60 % of their calories from fat displayed lower glucose disposal in response to insulin (Kang et al. 2014). These studies have highlighted the in vivo relationship between ECM expansion and muscle insulin resistance that may have otherwise been neglected if a dietary challenge was not performed. Furthermore, they effectively present the value of provocative techniques in determining variations in glucoregulation among experimental groups.

Energy balance

Obesity is a major public health problem in the Western world. A great deal has been learned from the mouse about the interaction of genetics and environment in obesity. Weight gain occurs when energy (i.e., nutrients, calories) intake exceeds energy expenditure. Energy expenditure is equal to the sum of internal heat produced, external work, and energy stored. It then follows that weight loss occurs when energy intake is less than energy expenditure. The mouse is weight stable when energy intake equals energy expenditure. The first indication that studies of energy balance may be informative is if there are differences in body weight or composition between groups. There are outstanding products on the market that measure the components of energy balance with great precision in singularly housed mice. These technologies have the potential to simultaneously measure food intake, fluid intake, activity in three planes, body weight, CO_2 output, and O_2 uptake. Energy expenditure and the respiratory exchange ratio are calculated from CO_2 output and O_2 uptake. Feeding behavior can also be characterized by the measurement of frequency and duration of feeding, and food preferences.

The measurement of energy expenditure has been fraught with controversy in recent years. Himms-Hagen (1997) and Butler and Kozak (2010) pointed out a recurring problem with the analysis of energy expenditure in genetic models comparing lean and obese phenotypes. It had become standard practice to normalize energy expenditure by total body weight. This makes little difference when comparing mice of similar body weight and identical body composition. However, it becomes a difficult problem when comparing obese mice such as the *ob/ob* to lean *ob/+* mice (Himms-Hagen 1997). The obese mice will have a lower energy expenditure when normalized per total body weight, even if there are no thermodynamic differences in the composite tissues. In fact, they showed that results could yield a range of conclusions based on how data were normalized (e.g., per mouse, per total lean mass, per total body mass). A more accurate and more tractable approach of normalizing to total lean mass was proposed and is regularly used (Butler and Kozak 2010). However, this approach was shown not to be altogether accurate as fat mass contributes significantly to energy expenditure (Kaiyala et al. 2010). Analysis by Kaiyala and Schwartz (2011) emphasized that any ratio-based method for normalizing energy expenditure to body mass compartments (whether total body mass or lean body mass is utilized) can lead to confounded outcomes. This prompted multiple linear regression to be recommended for use to control for variation in body mass and composition in mouse studies where energy expenditure is an important outcome variable (Kaiyala and Schwartz 2011). The NIH Mouse Metabolic Phenotyping Centers have sponsored the development of a portal for calculating energy expenditure using multiple linear regression. This portal was developed by Drs. Karl Kaiyala, Charles Speakerman, and Robert Podalsky and is accessible to the scientific community. (http://www.mmpc.org/shared/regression.aspx).

Conclusion

A vast number of mouse models displaying a spectrum of metabolic phenotypes have been studied and are available to be studied. Additionally, technologies to assess insulin action, insulin secretion, glucose metabolism, and energy balance have been developed and continue to be refined. Many of these approaches have been used in humans and are being scaled down to become compatible with the mouse. The biggest obstacle for laboratories wishing to conduct studies in the conscious mouse is to alleviate the confounding variables associated with stress by developing methods to avoid animal handling (Ayala et al. 2010). Sensitive analytical advancements in measurements of nutrient metabolism will be transformative in the field and accelerate our

understanding of metabolism. We hope that highlighting a few of the more recent trends in metabolic testing it becomes readily apparent that this is an exciting time for researchers in the field of metabolic diseases.

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References

Ae Park S, Choi MS, Cho SY, Seo JS, Jung UJ, Kim MJ, Sung MK, Park YB, Lee MK (2006) Genistein and daidzein modulate hepatic glucose and lipid regulating enzyme activities in C57BL/KsJ-db/db mice. Life Sci 79:1207–1213

Aigner B, Rathkolb B, Herbach N, Hrabe de Angelis M, Wanke R, Wolf E (2008) Diabetes models by screen for hyperglycemia in phenotype-driven ENU mouse mutagenesis projects. Am J Physiol Endocrinol Metab 294:E232–E240

Akinmokun A, Selby PL, Ramaiya K, Alberti KG (1992) The short insulin tolerance test for determination of insulin sensitivity: a comparison with the euglycemic clamp. Diabet Med 9:432–437

Albarado DC, McClaine J, Stephens JM, Mynatt RL, Ye J, Bannon AW, Richards WG, Butler AA (2004) Impaired coordination of nutrient intake and substrate oxidation in melanocortin-4 receptor knockout mice. Endocrinology 145:243–252

Alexander J, Chang GQ, Dourmashkin JT, Leibowitz SF (2006) Distinct phenotypes of obesity-prone AKR/J, DBA2J and C57BL/6J mice compared to control strains. Int J Obes 30:50–59

Alonso LC, Watanabe Y, Stefanovski D, Lee EJ, Singamsetty S, Romano LC, Zou B, Garcia-Ocana A, Bergman RN, O'Donnell CP (2012) Simultaneous measurement of insulin sensitivity, insulin secretion, and the disposition index in conscious unhandled mice. Obes (Silver Spring) 20:1403–1412

Anstee QM, Goldin RD (2006) Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. Int J Exp Pathol 87:1–16

Antoniewicz MR, Kelleher JK, Stephanopoulos G (2011) Measuring deuterium enrichment of glucose hydrogen atoms by gas chromatography/mass spectrometry. Anal Chem 83:3211–3216

Araki E, Lipes MA, Patti ME, Bruning JC, Haag B 3rd, Johnson RS, Kahn CR (1994) Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. Nature 372:186–190

Ayala JE, Bracy DP, McGuinness OP, Wasserman DH (2006) Considerations in the design of hyperinsulinemic-euglycemic clamps in the conscious mouse. Diabetes 55:390–397

Ayala JE, Samuel VT, Morton GJ, Obici S, Croniger CM, Shulman GI, Wasserman DH, McGuinness OP (2010) Standard operating procedures for describing and performing metabolic tests of glucose homeostasis in mice. Dis Models Mech 3:525–534

Ayala JE, Bracy DP, Malabanan C, James FD, Ansari T, Fueger PT, McGuinness OP, Wasserman DH (2011) Hyperinsulinemic-euglycemic clamps in conscious, unrestrained mice. J Vis Exp 57:3188

Bain JR, Stevens RD, Wenner BR, Ilkayeva O, Muoio DM, Newgard CB (2009) Metabolomics applied to diabetes research: moving from information to knowledge. Diabetes 58:2429–2443

Basciano H, Federico L, Adeli K (2005) Fructose, insulin resistance, and metabolic dyslipidemia. Nutr Metab 2:5

Berglund ED, Li CY, Poffenberger G, Ayala JE, Fueger PT, Willis SE, Jewell MM, Powers AC, Wasserman DH (2008) Glucose metabolism in vivo in four commonly used inbred mouse strains. Diabetes 57:1790–1799

Bergman RN, Phillips LS, Cobelli C (1981) Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. J Clin Investig 68:1456–1467

Bigorgne AE, Bouchet-Delbos L, Naveau S, Dagher I, Prevot S, Durand-Gasselin I, Couderc J, Valet P, Emilie D, Perlemuter G (2008) Obesity-induced lymphocyte hyperresponsiveness to chemokines: a new mechanism of Fatty liver inflammation in obese mice. Gastroenterology 134:1459–1469

Bonner JS, Lantier L, Hasenour CM, James FD, Bracy DP, Wasserman DH (2013) Muscle-specific vascular endothelial growth factor deletion induces muscle capillary rarefaction creating muscle insulin resistance. Diabetes 62:572–580

Bray GA, Nielsen SJ, Popkin BM (2004) Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. Am J Clin Nutr 79:537–543

Bruning JC, Winnay J, Bonner-Weir S, Taylor SI, Accili D, Kahn CR (1997) Development of a novel polygenic model of NIDDM in mice heterozygous for IR and IRS-1 null alleles. Cell 88:561–572

Bruning JC, Michael MD, Winnay JN, Hayashi T, Horsch D, Accili D, Goodyear LJ, Kahn CR (1998) A muscle-specific insulin receptor knockout exhibits features of the metabolic syndrome of NIDDM without altering glucose tolerance. Mol Cell 2:559–569

Buettner R, Scholmerich J, Bollheimer LC (2007) High-fat diets: modeling the metabolic disorders of human obesity in rodents. Obesity 15:798–808

Burgess SC, Jeffrey FM, Storey C, Milde A, Hausler N, Merritt ME, Mulder H, Holm C, Sherry AD, Malloy CR (2005) Effect of murine strain on metabolic pathways of glucose production after brief or prolonged fasting. Am J Physiol Endocrinol Metab 289:E53–E61

Butler AA, Kozak LP (2010) A recurring problem with the analysis of energy expenditure in genetic models expressing lean and obese phenotypes. Diabetes 59:323–329

Butler AA, Kesterson RA, Khong K, Cullen MJ, Pelleymounter MA, Dekoning J, Baetscher M, Cone RD (2000) A unique metabolic syndrome causes obesity in the melanocortin-3 receptor-deficient mouse. Endocrinology 141:3518–3521

Camporez JP, Jornayvaz FR, Lee HY, Kanda S, Guigni BA, Kahn M, Samuel VT, Carvalho CR, Petersen KF, Jurczak MJ, Shulman GI (2013) Cellular mechanism by which estradiol protects female ovariectomized mice from high-fat diet-induced hepatic and muscle insulin resistance. Endocrinology 154:1021–1028

Castle AL, Fiehn O, Kaddurah-Daouk R, Lindon JC (2006) Metabolomics Standards Workshop and the development of international standards for reporting metabolomics experimental results. Brief Bioinform 7:159–165

Chen AS, Marsh DJ, Trumbauer ME, Frazier EG, Guan XM, Yu H, Rosenblum CI, Vongs A, Feng Y, Cao L, Metzger JM, Strack AM, Camacho RE, Mellin TN, Nunes CN, Min W, Fisher J, Gopal-Truter S, MacIntyre DE, Chen HY, Van der Ploeg LH (2000) Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. Nat Genet 26:97–102

Chicco A, D'Alessandro ME, Karabatas L, Pastorale C, Basabe JC, Lombardo YB (2003) Muscle lipid metabolism and insulin secretion are altered in insulin-resistant rats fed a high sucrose diet. J Nutr 133:127–133

Coleman DL, Hummel KP (1973) The influence of genetic background on the expression of the obese (Ob) gene in the mouse. Diabetologia 9:287–293

Cone RD (2005) Anatomy and regulation of the central melanocortin system. Nat Neurosci 8:571–578

Cresto JC, Lavine RL, Buchly ML, Penhos JC, Bhathena SJ, Recant L (1977) Half life of injected 125I-insulin in control and ob/ob mice. Acta Physiol Lat Am 27:7–15

Dickie MM (1969) Mutations at the agouti locus in the mouse. J Hered 60:20–25

Dobrzyn P, Dobrzyn A, Miyazaki M, Ntambi JM (2010) Loss of stearoyl-CoA desaturase 1 rescues cardiac function in obese leptin-deficient mice. J Lipid Res 51:2202–2210

Dong YF, Liu L, Kataoka K, Nakamura T, Fukuda M, Tokutomi Y, Nako H, Ogawa H, Kim-Mitsuyama S (2010) Aliskiren prevents cardiovascular complications and pancreatic injury in a mouse model of obesity and type 2 diabetes. Diabetologia 53:180–191

Dubuc PU (1976) The development of obesity, hyperinsulinemia, and hyperglycemia in ob/ob mice. Metabolism 25:1567–1574

Ellacott KL, Murphy JG, Marks DL, Cone RD (2007) Obesity-induced inflammation in white adipose tissue is attenuated by loss of melanocortin-3 receptor signaling. Endocrinology 148:6186–6194

Enser M (1972) Clearing-factor lipase in obese hyperglycaemic mice (ob–ob). Biochem J 129:447–453

Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD (1997) Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature 385:165–168

Farooqi IS, Keogh JM, Yeo GS, Lank EJ, Cheetham T, O'Rahilly S (2003) Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. New Engl J Med 348:1085–1095

Ge F, Zhou S, Hu C, Lobdell Ht, Berk PD (2010) Insulin- and leptin-regulated fatty acid uptake plays a key causal role in hepatic steatosis in mice with intact leptin signaling but not in ob/ob or db/db mice. American journal of physiology. Gastrointest Liv Physiol 299:G855–G866

Goldsworthy M, Absalom NL, Schroter D, Matthews HC, Bogani D, Moir L, Long A, Church C, Hugill A, Anstee QM, Goldin R, Thursz M, Hollfelder F, Cox RD (2013) Mutations in Mll2, an H3K4 methyltransferase, result in insulin resistance and impaired glucose tolerance in mice. PLoS ONE 8:e61870

Goren HJ, Kulkarni RN, Kahn CR (2004) Glucose homeostasis and tissue transcript content of insulin signaling intermediates in four inbred strains of mice: c57BL/6, C57BLKS/6, DBA/2, and 129X1. Endocrinology 145:3307–3323

Guo S (2014) Insulin signaling, resistance, and the metabolic syndrome: insights from mouse models into disease mechanisms. J Endocrinol 220:T1–T23

Han BG, Hao CM, Tchekneva EE, Wang YY, Lee CA, Ebrahim B, Harris RC, Kern TS, Wasserman DH, Breyer MD, Qi Z (2008) Markers of glycemic control in the mouse: comparisons of 6-h- and overnight-fasted blood glucoses to Hb A1c. Am J Physiol Endocrinol Metab 295:E981–E986

Hasenour CM, Ridley DE, Hughey CC, James FD, Donahue EP, Shearer J, Viollet B, Foretz M, Wasserman DH (2014) 5-Aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR) effect on glucose production, but not energy metabolism, is independent of hepatic AMPK in vivo. J Biol Chem 289:5950–5959

Himms-Hagen J (1997) On raising energy expenditure in ob/ob mice. Science 276:1132–1133

Hinney A, Schmidt A, Nottebom K, Heibult O, Becker I, Ziegler A, Gerber G, Sina M, Gorg T, Mayer H, Siegfried W, Fichter M, Remschmidt H, Hebebrand J (1999) Several mutations in the melanocortin-4 receptor gene including a nonsense and a frameshift mutation associated with dominantly inherited obesity in humans. J Clin Endocrinol Metab 84:1483–1486

Hirschey MD, Shimazu T, Goetzman E, Jing E, Schwer B, Lombard DB, Grueter CA, Harris C, Biddinger S, Ilkayeva OR, Stevens RD, Li Y, Saha AK, Ruderman NB, Bain JR, Newgard CB, Farese RV Jr, Alt FW, Kahn CR, Verdin E (2010) SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. Nature 464:121–125

Hong CJ, Tsai PJ, Cheng CY, Chou CK, Jheng HF, Chuang YC, Yang CN, Lin YT, Hsu CW, Cheng IH, Chen SY, Tsai SJ, Liou YJ, Tsai YS (2010) ENU mutagenesis identifies mice with morbid obesity and severe hyperinsulinemia caused by a novel mutation in leptin. PLoS ONE 5:e15333

Hummel KP, Dickie MM, Coleman DL (1966) Diabetes, a new mutation in the mouse. Science 153:1127–1128

Humphreys MH, Ni XP, Pearce D (2011) Cardiovascular effects of melanocortins. Eur J Pharmacol 660:43–52

Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, Smith FJ, Campfield LA, Burn P, Lee F (1997) Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88:131–141

Hynes RO (2002) Integrins: bidirectional, allosteric signaling machines. Cell 110:673–687

Ikemoto S, Takahashi M, Tsunoda N, Maruyama K, Itakura H, Ezaki O (1996) High-fat diet-induced hyperglycemia and obesity in mice: differential effects of dietary oils. Metabolism 45:1539–1546

Ingalls AM, Dickie MM, Snell GD (1950) Obese, a new mutation in the house mouse. J Hered 41:317–318

Inoue M, Sakuraba Y, Motegi H, Kubota N, Toki H, Matsui J, Toyoda Y, Miwa I, Terauchi Y, Kadowaki T, Shigeyama Y, Kasuga M, Adachi T, Fujimoto N, Matsumoto R, Tsuchihashi K, Kagami T, Inoue A, Kaneda H, Ishijima J, Masuya H, Suzuki T, Wakana S, Gondo Y, Minowa O, Shiroishi T, Noda T (2004) A series of maturity onset diabetes of the young, type 2 (MODY2) mouse models generated by a large-scale ENU mutagenesis program. Hum Mol Genet 13:1147–1157

Jackerott M, Baudry A, Lamothe B, Bucchini D, Jami J, Joshi RL (2001) Endocrine pancreas in insulin receptor-deficient mouse pups. Diabetes 50(Suppl 1):S146–S149

Jacobson L, Ansari T, McGuinness OP (2006a) Counterregulatory deficits occur within 24 h of a single hypoglycemic episode in conscious, unrestrained, chronically cannulated mice. American journal of physiology. Endocrinol Metab 290:E678–E684

Jacobson L, Ansari T, Potts J, McGuinness OP (2006b) Glucocorticoid-deficient corticotropin-releasing hormone knockout mice maintain glucose requirements but not autonomic responses during repeated hypoglycemia. American journal of physiology. Endocrinol Metab 291:E15–E22

James DE, Burleigh KM, Chisholm DJ, Kraegen EW (1985) In vivo dose response curves of insulin action in heart: anomalous effects at high insulin doses. J Mol Cell Cardiol 17:981–985

Jin ES, Jones JG, Merritt M, Burgess SC, Malloy CR, Sherry AD (2004) Glucose production, gluconeogenesis, and hepatic tricarboxylic acid cycle fluxes measured by nuclear magnetic resonance analysis of a single glucose derivative. Anal Biochem 327:149–155

Jin ES, Beddow SA, Malloy CR, Samuel VT (2013) Hepatic glucose production pathways after three days of a high-fat diet. Metabolism 62:152–162

Jones JG, Naidoo R, Sherry AD, Jeffrey FM, Cottam GL, Malloy CR (1997) Measurement of gluconeogenesis and pyruvate recycling in the rat liver: a simple analysis of glucose and glutamate isotopomers during metabolism of [1,2,3-(13)C3]propionate. FEBS Lett 412:131–137

Jones JG, Solomon MA, Cole SM, Sherry AD, Malloy CR (2001) An integrated (2)H and (13)C NMR study of gluconeogenesis and TCA cycle flux in humans. Am J Physiol Endocrinol Metab 281:E848–E856

Jourdan T, Djaouti L, Demizieux L, Gresti J, Verges B, Degrace P (2009) Liver carbohydrate and lipid metabolism of insulin-deficient mice is altered by trans-10, cis-12 conjugated linoleic acid. J Nutr 139:1901–1907

Kaiyala KJ, Schwartz MW (2011) Toward a more complete (and less controversial) understanding of energy expenditure and its role in obesity pathogenesis. Diabetes 60:17–23

Kaiyala KJ, Morton GJ, Leroux BG, Ogimoto K, Wisse B, Schwartz MW (2010) Identification of body fat mass as a major determinant of metabolic rate in mice. Diabetes 59:1657–1666

Kang L, Ayala JE, Lee-Young RS, Zhang Z, James FD, Neufer PD, Pozzi A, Zutter MM, Wasserman DH (2011) Diet-induced muscle insulin resistance is associated with extracellular matrix remodeling and interaction with integrin alpha2beta1 in mice. Diabetes 60:416–426

Kang L, Mayes WH, James FD, Bracy DP, Wasserman DH (2014) Matrix metalloproteinase 9 opposes diet-induced muscle insulin resistance in mice. Diabetologia 57:603–613

Katz EB, Stenbit AE, Hatton K, DePinho R, Charron MJ (1995) Cardiac and adipose tissue abnormalities but not diabetes in mice deficient in GLUT4. Nature 377:151–155

Kennedy AJ, Ellacott KL, King VL, Hasty AH (2010) Mouse models of the metabolic syndrome. Dis Models Mech 3:156–166

Kido Y, Burks DJ, Withers D, Bruning JC, Kahn CR, White MF, Accili D (2000) Tissue-specific insulin resistance in mice with mutations in the insulin receptor, IRS-1, and IRS-2. J Clin Investig 105:199–205

Kobayasi R, Akamine EH, Davel AP, Rodrigues MA, Carvalho CR, Rossoni LV (2010) Oxidative stress and inflammatory mediators contribute to endothelial dysfunction in high-fat diet-induced obesity in mice. J Hypertens 28:2111–2119

Kraegen EW, James DE, Jenkins AB, Chisholm DJ (1985) Dose-response curves for in vivo insulin sensitivity in individual tissues in rats. Am J Physiol 248:E353–E362

Kushner JA, Ye J, Schubert M, Burks DJ, Dow MA, Flint CL, Dutta S, Wright CV, Montminy MR, White MF (2002) Pdx1 restores beta cell function in Irs2 knockout mice. J Clin Investig 109:1193–1201

Lam TK, Gutierrez-Juarez R, Pocai A, Bhanot S, Tso P, Schwartz GJ, Rossetti L (2007) Brain glucose metabolism controls the hepatic secretion of triglyceride-rich lipoproteins. Nat Med 13:171–180

Laughlin MR, Lloyd KC, Cline GW, Wasserman DH (2012) NIH Mouse Metabolic Phenotyping Centers: the power of centralized phenotyping. Mamm Genome 23:623–631

Lee AW, Hengstler H, Schwald K, Berriel-Diaz M, Loreth D, Kirsch M, Kretz O, Haas CA, de Angelis MH, Herzig S, Brummendorf T, Klingenspor M, Rathjen FG, Rozman J, Nicholson G, Cox RD, Schafer MK (2012) Functional inactivation of the genome-wide association study obesity gene neuronal growth regulator 1 in mice causes a body mass phenotype. PLoS ONE 7:e41537

Lee-Young RS, Ayala JE, Hunley CF, James FD, Bracy DP, Kang L, Wasserman DH (2010) Endothelial nitric oxide synthase is central to skeletal muscle metabolic regulation and enzymatic signaling during exercise in vivo. Am J Physiol Regul Integr Comp Physiol 298:R1399–R1408

Lenzen S (2008) The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia 51:216–226

Leto D, Saltiel AR (2012) Regulation of glucose transport by insulin: traffic control of GLUT4. Nat Rev Mol Cell Biol 13:383–396

Lim JS, Mietus-Snyder M, Valente A, Schwarz JM, Lustig RH (2010) The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. Nat Rev Gastroenterol Hepatol 7:251–264

Lu D, Willard D, Patel IR, Kadwell S, Overton L, Kost T, Luther M, Chen W, Woychik RP, Wilkison WO et al (1994) Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. Nature 371:799–802

Mark AL, Shaffer RA, Correia ML, Morgan DA, Sigmund CD, Haynes WG (1999) Contrasting blood pressure effects of obesity in leptin-deficient ob/ob mice and agouti yellow obese mice. J Hypertens 17:1949–1953

Marti A, Corbalan MS, Forga L, Martinez JA, Hinney A, Hebebrand J (2003) A novel nonsense mutation in the melanocortin-4 receptor associated with obesity in a Spanish population. Int J Obes Relat Metab Disord 27:385–388

Massiera F, Barbry P, Guesnet P, Joly A, Luquet S, Moreilhon-Brest C, Mohsen-Kanson T, Amri EZ, Ailhaud G (2010) A Western-like fat diet is sufficient to induce a gradual enhancement in fat mass over generations. J Lipid Res 51:2352–2361

McGuinness OP, Ayala JE, Laughlin MR, Wasserman DH (2009) NIH experiment in centralized mouse phenotyping: the Vanderbilt experience and recommendations for evaluating glucose homeostasis in the mouse. American journal of physiology. Endocrinol Metab 297:E849–E855

Mergen M, Mergen H, Ozata M, Oner R, Oner C (2001) A novel melanocortin 4 receptor (MC4R) gene mutation associated with morbid obesity. J Clin Endocrinol Metab 86:3448

Meyre D, Delplanque J, Chevre JC, Lecoeur C, Lobbens S, Gallina S, Durand E, Vatin V, Degraeve F, Proenca C, Gaget S, Korner A, Kovacs P, Kiess W, Tichet J, Marre M, Hartikainen AL, Horber F, Potoczna N, Hercberg S, Levy-Marchal C, Pattou F, Heude B, Tauber M, McCarthy MI, Blakemore AI, Montpetit A, Polychronakos C, Weill J, Coin LJ, Asher J, Elliott P, Jarvelin MR, Visvikis-Siest S, Balkau B, Sladek R, Balding D, Walley A, Dina C, Froguel P (2009) Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. Nat Genet 41:157–159

Morton GJ, Matsen ME, Bracy DP, Meek TH, Nguyen HT, Stefanovski D, Bergman RN, Wasserman DH, Schwartz MW (2013) FGF19 action in the brain induces insulin-independent glucose lowering. J Clin Investig 123:4799–4808

Nagase H, Visse R, Murphy G (2006) Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res 69:562–573

Nagata R, Nishio Y, Sekine O, Nagai Y, Maeno Y, Ugi S, Maegawa H, Kashiwagi A (2004) Single nucleotide polymorphism (−468 Gly to A) at the promoter region of SREBP-1c associates with genetic defect of fructose-induced hepatic lipogenesis [corrected]. J Biol Chem 279:29031–29042

Nandi A, Kitamura Y, Kahn CR, Accili D (2004) Mouse models of insulin resistance. Physiol Rev 84:623–647

Nishina PM, Lowe S, Wang J, Paigen B (1994) Characterization of plasma lipids in genetically obese mice: the mutants obese, diabetes, fat, tubby, and lethal yellow. Metabolism 43:549–553

Nogueiras R, Wiedmer P, Perez-Tilve D, Veyrat-Durebex C, Keogh JM, Sutton GM, Pfluger PT, Castaneda TR, Neschen S, Hofmann SM, Howles PN, Morgan DA, Benoit SC, Szanto I, Schrott B, Schurmann A, Joost HG, Hammond C, Hui DY, Woods SC, Rahmouni K, Butler AA, Farooqi IS, O'Rahilly S, Rohner-Jeanrenaud F, Tschop MH (2007) The central melanocortin system directly controls peripheral lipid metabolism. J Clin Investig 117:3475–3488

Nunemaker CS, Zhang M, Wasserman DH, McGuinness OP, Powers AC, Bertram R, Sherman A, Satin LS (2005) Individual mice can be distinguished by the period of their islet calcium oscillations: is there an intrinsic islet period that is imprinted in vivo? Diabetes 54:3517–3522

Nunemaker CS, Wasserman DH, McGuinness OP, Sweet IR, Teague JC, Satin LS (2006) Insulin secretion in the conscious mouse is biphasic and pulsatile. American journal of physiology. Endocrinol Metab 290:E523–E529

Oosterveer MH, Mataki C, Yamamoto H, Harach T, Moullan N, van Dijk TH, Ayuso E, Bosch F, Postic C, Groen AK, Auwerx J, Schoonjans K (2012) LRH-1-dependent glucose sensing determines intermediary metabolism in liver. J Clin Invest 122:2817–2826

Osborn O, Sanchez-Alavez M, Brownell SE, Ross B, Klaus J, Dubins J, Beutler B, Conti B, Bartfai T (2010) Metabolic characterization of a mouse deficient in all known leptin receptor isoforms. Cell Mol Neurobiol 30:23–33

Paik SG, Fleischer N, Shin SI (1980) Insulin-dependent diabetes mellitus induced by subdiabetogenic doses of streptozotocin: obligatory role of cell-mediated autoimmune processes. Proc Natl Acad Sci USA 77:6129–6133

Palm F, Ortsater H, Hansell P, Liss P, Carlsson PO (2004) Differentiating between effects of streptozotocin per se and subsequent hyperglycemia on renal function and metabolism in the streptozotocin-diabetic rat model. Diabetes/Metab Res Rev 20:452–459

Panchal SK, Brown L (2011) Rodent models for metabolic syndrome research. J Biomed Biotechnol 2011:351982

Park HJ, DiNatale DA, Chung MY, Park YK, Lee JY, Koo SI, O'Connor M, Manautou JE, Bruno RS (2011) Green tea extract attenuates hepatic steatosis by decreasing adipose lipogenesis and enhancing hepatic antioxidant defenses in ob/ob mice. J Nutr Biochem 22:393–400

Plumpton FS, Besser GM (1969) The adrenocortical response to surgery and insulin-induced hypoglycaemia in corticosteroid-treated and normal subjects. Br J Surg 56:216–219

Preitner F, Mody N, Graham TE, Peroni OD, Kahn BB (2009) Long-term Fenretinide treatment prevents high-fat diet-induced obesity, insulin resistance, and hepatic steatosis. Am J Physiol Endocrinol Metab 297:E1420–E1429

Previs SF, Fernandez CA, Yang D, Soloviev MV, David F, Brunengraber H (1995) Limitations of the mass isotopomer distribution analysis of glucose to study gluconeogenesis. Substrate cycling between glycerol and triose phosphates in liver. J Biol Chem 270:19806–19815

Sahai A, Malladi P, Pan X, Paul R, Melin-Aldana H, Green RM, Whitington PF (2004) Obese and diabetic db/db mice develop marked liver fibrosis in a model of nonalcoholic steatohepatitis: role of short-form leptin receptors and osteopontin. American journal of physiology. Gastrointest Liv Physiol 287:G1035–G1043

Satapati S, Sunny NE, Kucejova B, Fu X, He TT, Mendez-Lucas A, Shelton JM, Perales JC, Browning JD, Burgess SC (2012) Elevated TCA cycle function in the pathology of diet-induced hepatic insulin resistance and fatty liver. J Lipid Res 53:1080–1092

Schein PS, Loftus S (1968) Streptozotocin: depression of mouse liver pyridine nucleotides. Cancer Res 28:1501–1506

Sharma K, Kohli P, Gulati M (2012) An update on exercise stress testing. Curr Probl Cardiol 37:177–202

Slot JW, Geuze HJ, Gigengack S, James DE, Lienhard GE (1991) Translocation of the glucose transporter GLUT4 in cardiac myocytes of the rat. Proc Natl Acad Sci U S A 88:7815–7819

Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M (1977) The [14C]deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem 28:897–916

Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P (2005) Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res 52:313–320

Ste Marie L, Miura GI, Marsh DJ, Yagaloff K, Palmiter RD (2000) A metabolic defect promotes obesity in mice lacking melanocortin-4 receptors. Proc Natl Acad Sci USA 97:12339–12344

Stenbit AE, Tsao TS, Li J, Burcelin R, Geenen DL, Factor SM, Houseknecht K, Katz EB, Charron MJ (1997) GLUT4 heterozygous knockout mice develop muscle insulin resistance and diabetes. Nat Med 3:1096–1101

Sumiyoshi M, Sakanaka M, Kimura Y (2006) Chronic intake of high-fat and high-sucrose diets differentially affects glucose intolerance in mice. J Nutr 136:582–587

Sunny NE, Parks EJ, Browning JD, Burgess SC (2011) Excessive hepatic mitochondrial TCA cycle and gluconeogenesis in humans with nonalcoholic fatty liver disease. Cell Metab 14:804–810

Surwit RS, Kuhn CM, Cochrane C, McCubbin JA, Feinglos MN (1988) Diet-induced type II diabetes in C57BL/6 J mice. Diabetes 37:1163–1167

Sutton GM, Trevaskis JL, Hulver MW, McMillan RP, Markward NJ, Babin MJ, Meyer EA, Butler AA (2006) Diet-genotype interactions in the development of the obese, insulin-resistant phenotype of C57BL/6 J mice lacking melanocortin-3 or -4 receptors. Endocrinology 147:2183–2196

Svenson KL, Ahituv N, Durgin RS, Savage H, Magnani PA, Foreman O, Paigen B, Peters LL (2008) A new mouse mutant for the LDL receptor identified using ENU mutagenesis. J Lipid Res 49:2452–2462

Tallam LS, Stec DE, Willis MA, da Silva AA, Hall JE (2005) Melanocortin-4 receptor-deficient mice are not hypertensive or salt-sensitive despite obesity, hyperinsulinemia, and hyperleptinemia. Hypertension 46:326–332

Tamemoto H, Kadowaki T, Tobe K, Yagi T, Sakura H, Hayakawa T, Terauchi Y, Ueki K, Kaburagi Y, Satoh S et al (1994) Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. Nature 372:182–186

Tran LT, Yuen VG, McNeill JH (2009) The fructose-fed rat: a review on the mechanisms of fructose-induced insulin resistance and hypertension. Mol Cell Biochem 332:145–159

Trevaskis JL, Meyer EA, Galgani JE, Butler AA (2008) Counterintuitive effects of double-heterozygous null melanocortin-4 receptor and leptin genes on diet-induced obesity and insulin resistance in C57BL/6 J mice. Endocrinology 149:174–184

Tschop M, Heiman ML (2001) Rodent obesity models: an overview. Exp Clin Endocrinol Diabet 109, 307–319

Vaisse C, Clement K, Guy-Grand B, Froguel P (1998) A frameshift mutation in human MC4R is associated with a dominant form of obesity. Nat Genet 20:113–114

van Burck L, Blutke A, Kautz S, Rathkolb B, Klaften M, Wagner S, Kemter E, Hrabe de Angelis M, Wolf E, Aigner B, Wanke R, Herbach N (2010) Phenotypic and pathomorphological characteristics of a novel mutant mouse model for maturity-onset diabetes of the young type 2 (MODY 2). Am J Physiol Endocrinol Metab 298:E512–E523

Van den Bergh A, Vanderper A, Vangheluwe P, Desjardins F, Nevelsteen I, Verreth W, Wuytack F, Holvoet P, Flameng W, Balligand JL, Herijgers P (2008) Dyslipidaemia in type II diabetic mice does not aggravate contractile impairment but increases ventricular stiffness. Cardiovasc Res 77:371–379

Vella A, Rizza RA (2009) Application of isotopic techniques using constant specific activity or enrichment to the study of carbohydrate metabolism. Diabetes 58:2168–2174

Wang H, Storlien LH, Huang XF (2002) Effects of dietary fat types on body fatness, leptin, and ARC leptin receptor, NPY, and AgRP mRNA expression. Am J Physiol Endocrinol Metab 282:E1352–E1359

Wasserman DH (2009) Four grams of glucose. Am J Physiol Endocrinol Metab 296:E11–E21

Wasserman DH, Ayala JE, McGuinness OP (2009) Lost in translation. Diabetes 58:1947–1950

Willer CJ, Speliotes EK, Loos RJ, Li S, Lindgren CM, Heid IM, Berndt SI, Elliott AL, Jackson AU, Lamina C, Lettre G, Lim N, Lyon HN, McCarroll SA, Papadakis K, Qi L, Randall JC, Roccasecca RM, Sanna S, Scheet P, Weedon MN, Wheeler E, Zhao JH, Jacobs LC, Prokopenko I, Soranzo N, Tanaka T, Timpson NJ, Almgren P, Bennett A, Bergman RN, Bingham SA, Bonnycastle LL, Brown M, Burtt NP, Chines P, Coin L, Collins

FS, Connell JM, Cooper C, Smith GD, Dennison EM, Deodhar P, Elliott P, Erdos MR, Estrada K, Evans DM, Gianniny L, Gieger C, Gillson CJ, Guiducci C, Hackett R, Hadley D, Hall AS, Havulinna AS, Hebebrand J, Hofman A, Isomaa B, Jacobs KB, Johnson T, Jousilahti P, Jovanovic Z, Khaw KT, Kraft P, Kuokkanen M, Kuusisto J, Laitinen J, Lakatta EG, Luan J, Luben RN, Mangino M, McArdle WL, Meitinger T, Mulas A, Munroe PB, Narisu N, Ness AR, Northstone K, O'Rahilly S, Purmann C, Rees MG, Ridderstrale M, Ring SM, Rivadeneira F, Ruokonen A, Sandhu MS, Saramies J, Scott LJ, Scuteri A, Silander K, Sims MA, Song K, Stephens J, Stevens S, Stringham HM, Tung YC, Valle TT, Van Duijn CM, Vimaleswaran KS, Vollenweider P, Waeber G, Wallace C, Watanabe RM, Waterworth DM, Watkins N, Witteman JC, Zeggini E, Zhai G, Zillikens MC, Altshuler D, Caulfield MJ, Chanock SJ, Farooqi IS, Ferrucci L, Guralnik JM, Hattersley AT, Hu FB, Jarvelin MR, Laakso M, Mooser V, Ong KK, Ouwehand WH, Salomaa V, Samani NJ, Spector TD, Tuomi T, Tuomilehto J, Uda M, Uitterlinden AG, Wareham NJ, Deloukas P, Frayling TM, Groop LC, Hayes RB, Hunter DJ, Mohlke KL, Peltonen L, Schlessinger D, Strachan DP, Wichmann HE, McCarthy MI, Boehnke M, Barroso I, Abecasis GR, Hirschhorn JN (2009) Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet 41:25–34

Williams TD, Chambers JB, Gagnon SP, Roberts LM, Henderson RP, Overton JM (2003) Cardiovascular and metabolic responses to fasting and thermoneutrality in Ay mice. Physiol Behav 78:615–623

Winzell MS, Wulff EM, Olsen GS, Sauerberg P, Gotfredsen CF, Ahren B (2010) Improved insulin sensitivity and islet function after PPARdelta activation in diabetic db/db mice. Eur J Pharmacol 626:297–305

Withers DJ, Burks DJ, Towery HH, Altamuro SL, Flint CL, White MF (1999) Irs-2 coordinates Igf-1 receptor-mediated beta-cell development and peripheral insulin signalling. Nat Genet 23:32–40

Yamauchi R, Kobayashi M, Matsuda Y, Ojika M, Shigeoka S, Yamamoto Y, Tou Y, Inoue T, Katagiri T, Murai A, Horio F (2010) Coffee and caffeine ameliorate hyperglycemia, fatty liver, and inflammatory adipocytokine expression in spontaneously diabetic KK-Ay mice. J Agric Food Chem 58:5597–5603

Yen TT, Gill AM, Frigeri LG, Barsh GS, Wolff GL (1994) Obesity, diabetes, and neoplasia in yellow A(vy)/- mice: ectopic expression of the agouti gene. FASEB J 8:479–488

Yeo GS, Farooqi IS, Aminian S, Halsall DJ, Stanhope RG, O'Rahilly S (1998) A frameshift mutation in MC4R associated with dominantly inherited human obesity. Nat Genet 20:111–112

Zaman AK, Fujii S, Goto D, Furumoto T, Mishima T, Nakai Y, Dong J, Imagawa S, Sobel BE, Kitabatake A (2004) Salutary effects of attenuation of angiotensin II on coronary perivascular fibrosis associated with insulin resistance and obesity. J Mol Cell Cardiol 37:525–535

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432