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Modelling insulin resistance in rodents by alterations in diet. What have "high fat" and high calorie diets revealed?

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Abstract

For over half a century, researchers have been feeding different diets to rodents to examine the effects of macronutrients on whole body and tissue insulin action. During this period, the number of different diets and the source of macronutrients employed has grown dramatically. Due to the large heterogeneity in both the source and percentage of different macronutrients used for studies, it is not surprising that different high calorie diets do not produce the same changes in insulin action. Despite this, diverse high calorie diets continue to be employed in an attempt to generate a "generic" insulin resistance. The "high fat diet" in particular varies greatly between studies with regard to the source, complexity and ratio of dietary fat, carbohydrate and protein. This review examines the range of rodent dietary models and methods for assessing insulin action. In almost all studies reviewed rodents fed diets that had more than 45% of dietary energy as fat or simple carbohydrates had reduced whole-body insulin action compared to chow. However, different high calorie diets produced significantly different effects in liver, muscle and whole-body insulin action when insulin action was measured by the hyperinsulinemic-euglycemic clamp method. Rodent dietary models remain an important tool for exploring potential mechanisms of insulin resistance but more attention needs to be given to the total macronutrient content and composition when interpreting dietary effects on insulin action.

Introduction

Metabolic conditions associated with nutrient overconsumption such as obesity, insulin resistance, type 2 diabetes, non-alcoholic steatohepatitis (NASH) and dyslipidemia present major challenges for the healthcare sector. Basic and clinical research that advances knowledge in this field is necessary for our understanding of metabolic disease. Animal models, and in particular rodent models, play a crucial role in the study of the complex pathologies caused by nutrient overconsumption and obesity. Although there are many surrogate measures of insulin action including glucose tolerance tests (GTT), insulin tolerance tests (ITT), HOMA-IR and insulin signalling phospho-immunoblots, hyperinsulinemic-euglycemic clamp data is considered the most robust way to directly compare insulin sensitivity within and between studies in animals and humans. This review examines the effect of diets on insulin action in rodent models of diet-induced obesity (DIO) by primarily reviewing investigations in which insulin action was directly measured systemically by the hyperinsulinemic-euglycemic clamp and/or peripherally by glucose tracer uptake at matched insulin levels. The review also focuses on liver and skeletal muscle which are considered responsible for the majority of insulin-stimulated glucose clearance in rodents and humans (84). This review complements and extends some excellent other reviews on dietary modulation of insulin action (156, 157) and on the rodent high-fat diet (HFD) model in general (21, 61).

Rodent models of insulin resistance

The most commonly used rodent models of obesity and the metabolic syndrome are either monogenic or diet induced. Monogenic models of obesity such as rodents with a defect in leptin production (ob/ob mice), or its receptor (db/db mice, (fa/fa) Zucker

fatty rats) (183) are extensively used as they generate animals with pronounced hyperphagia and consequently severe obesity. However, due to germ line deletions/mutations in these genes, these animals have many physiological differences from wild type rodents including alterations in the microvasculature (191), nervous system (50) and fertility problems (96). Therefore, the manifestations of metabolic dysfunction in leptin, or leptin receptor deficient rodents may not fully reflect metabolic disease aetiology in humans where congenital leptin deficiency is exceedingly rare (126). There are also several gene knockout mouse models of insulin resistance, mostly mice with deletions/mutations in genes coding for proteins in the insulin-signalling pathway or glucose transporters (111). While these models provide vital information for mechanistic studies they do not necessarily reflect the complexity and heterogeneity of the metabolic syndrome. Studies using polygenic models of rodent obesity where rodents are selectively bred to produce pronounced hyperphagia and obesity such as the New Zealand obese mouse (38, 131) and the Wellesley mouse (57) must also be interpreted with care for similar reasons and due to a lack of suitable "within strain" control animals to enable appropriate comparison between lean and obese animals. In contrast, the rodent DIO model has the advantage of having greater relevance to humans where overconsumption of calories invariably leads to increased fat mass and metabolic changes irrespective of genetic background (13). Additionally, the DIO model allows comparisons between genetically very similar or identical animals, which may reduce the potential variability of the study (51).

Tissue specific insulin action

Whole body glucose metabolism/insulin action is a sum of glucose metabolism in all the tissue of the body. However not all tissues of the body contribute to glucose utilisation to the same extent and not all tissues are regulated by insulin in the same way. Arguably the most important tissues to insulin-regulated glucose metabolism are the muscle, liver and adipose tissue.

Muscle

Impaired insulin-stimulated glucose uptake in skeletal muscle is one of the hallmarks of lipid induced insulin resistance. Skeletal muscle makes up 25-40% of body mass (76) and due to its relative size in man and most animals, muscle is considered to be a major tissue for the disposal of both glucose (74) and fatty acids (55). Under hyperinsulinemic-euglycemic clamp conditions, it has been calculated that skeletal muscle accounts for up to 80% of the total glucose disposal (44) and around 40% of glucose disposal after an oral glucose meal (73, 81). Skeletal muscle is also responsible for between 20 and 30% of resting energy expenditure (195). Additionally, muscle is capable of taking up and disposing of large amounts of glucose during and after exercise (137) which can have a major impact on glucose homeostasis and positive implications for metabolic health (17, 18).

There are multiple theories about the mechanisms that may be responsible for diet induced insulin resistance in muscle. Excess intramyocellular lipid (IMCL) content has been proposed to be a mediator as many reports show a strong negative correlation with whole body and skeletal muscle insulin sensitivity in humans and rodents (82, 89). However, this is not always the case (49) and interestingly, in athletes, who are very insulin sensitive, IMCL is high (the so-called athletes paradox (155)). Although an increase in lipid content is most clearly displayed as elevated

triacylglycerol (TAG) content, it is likely that TAGs may only act as a marker of dysfunctional muscle glucose metabolism. Accumulation of bioactive lipids or lipid intermediates such as diacylglycerol (DAGs), ceramides, acylcarnitines and/or acyl-CoAs is more likely to be involved in the observed decrease of insulin sensitivity (2, 12, 66, 72). Increased intracellular DAG levels resulting from fat feeding have been shown to activate protein kinase C (PKC) signalling leading to serine phosphorylation of IRS1. This prevents the phosphorylation of tyrosine residues on IRS1 leading to a defect in canonical PI3K/Akt signalling, reduced GLUT4 translocation and consequently reduced insulin-stimulated glucose uptake (52, 72, 193).

The DAG hypothesis of lipid-induced insulin resistance has gained considerable traction in the literature and has been reported to be present in the muscle of type 2 diabetic and lipid-infused humans (169). However, there are an increasing number of studies that provide conflicting evidence. These include genetic models with disruption of fatty acid metabolism leading to rodents which have increased muscle DAG concentration that retain (148) or even increase (173) muscle insulin sensitivity. Similar to DAG, another family of bioactive lipid species, ceramides, are also thought to inhibit insulin signalling (33), and have been shown to interact with several intracellular messengers including PKC isoforms, protein phosphatase 2A (PP2A) as well as kinases involved in inflammation pathways, JNK and NFκB (34, 163). Inhibiting ceramide synthesis has been shown to protect against glucocorticoid, DIO and saturated fat induced insulin resistance in rodents (66). Similar to the investigations in rodent models of insulin resistance, the human literature correlating different lipid species with insulin sensitivity in muscle is equally divided on the

143 importance of any specific lipids in the generation of insulin resistance (reviewed in
144 detail by Bosma *et al)* (12).
145
146 A dysfunction in the oxidative capacity of the mitochondria has also been suggested
147 as a mechanism underlying skeletal muscle insulin resistance (175). However other
148 reports show no differences in mitochondrial function, or indeed an increase in DIO
149 rodent models and between obese and lean humans (45, 176). A build-up of muscle
150 reactive oxygen species (ROS) and activation of inflammatory pathways by
151 circulating cytokines or intracellular lipids have also been proposed (186). Due to the
152 multiple levels of regulation of glucose uptake and disposal in muscle it is likely that
153 the range of muscle insulin sensitivity in humans may be due to a combination of
154 defects in extracellular glucose delivery (blood flow, microvascular recruitment),
155 transport (glucose transporter number, activity and translocation) and phosphorylation
156 (hexokinase activity) (185). Therefore, we believe that insulin action in muscle may
157 be negatively affected by multiple different metabolites/mechanisms as discussed
158 above. Clearly, our understanding of the mechanisms involved in diet-induced,
159 skeletal muscle insulin resistance remains incomplete despite the detailed analysis of
160 the molecular mechanisms involved in skeletal muscle insulin action outlined in
161 several review articles (1, 43, 69, 103, 142, 143, 177).
162
163 <u>Liver</u>
164 Hepatic insulin resistance, most commonly defined as the failure of insulin to supress
165 hepatic glucose production (HGP), is one of the first pathologies observed in DIO
166 models of insulin resistance (86, 141, 179). This is consistent with short term
167 overfeeding studies in humans that attributed a rapid loss of whole body insulin

sensitivity to defects in control of HGP (19, 36). The exact cellular mechanisms behind lipid induced hepatic insulin resistance are still debated, however it is clear that the development of non-alcoholic fatty liver disease (NAFLD) is strongly associated with a loss of the ability of insulin to inhibit glucose output. Similar to lipid-induced insulin resistance in muscle, one of the main theories suggested to explain this change in hepatic insulin sensitivity focuses on accumulation of bioactive lipid species. In this scenario the activation of PKCε by hepatic DAG accumulation, results in the inhibition of hepatic insulin signalling and subsequent increased expression and activation of gluconeogenic enzymes (127) although the time course for this sequence of events does not necessarily fit with the rapid ability (within minutes) of insulin to shut down glucose output from the liver. Liver ceramide content has also been implicated and has been reported to have a negative correlation with hepatic insulin action (118). Despite the many studies suggesting a causative role for liver lipid in systemic insulin resistance, some researchers have suggested that hepatic insulin resistance may precede NAFLD (53).

In addition to carbohydrate metabolism, the liver plays a crucial role in fatty acid metabolism. This includes the uptake, oxidation and *de novo* synthesis of fatty acids as well as packaging of lipids into lipoproteins for export and storage in other tissues (130). Lipogenesis and triglyceride synthesis in the liver are regulated by the transcription factor SREBP1c which is activated by insulin (15). In normal liver, stimulation of the insulin-signalling pathway leads to the activation of FOXO1 and SREBP1c leading to a transcriptional program to reduce glucose production and increase triglyceride synthesis. However, in the insulin resistant liver there is a loss of suppression of glucose production without decreases in triglyceride synthesis, despite

these processes being regulated by the same insulin-signalling pathway. This has led some researchers to the conclusion that there may be selective insulin resistance for different metabolic pathways in the liver (16). Due to the central role of the hepatocyte mitochondria in both the oxidation and synthesis of glucose and lipid, it is not surprising that mitochondrial function can be substantially altered in insulin resistant liver (37). Interestingly, in mouse models of NAFLD, there is a reported increase in the mitochondrial oxidative capacity of the liver (77, 78). Clearly the liver has a crucial role in regulating systemic glucose and fatty acid metabolism in both the fasting and postprandial state (84).

Adipose tissue

Although white adipose tissue (WAT) can contribute a significant portion to our body mass, particularly in obese individuals (54), it does not have a major impact on whole-body glucose disposal (54, 81, 88). In lean humans, adipose tissue is responsible for ~3% of the clearance of an oral glucose load (84). White adipose tissue also plays a relatively small role in the whole-body oxidation of fatty acids, instead it acts as more of a storage organ, releasing free fatty acids during fasting or starvation for use by other tissues such as heart and muscle (149). Despite its relative small contribution to glucose clearance, there is growing evidence that disruption of WAT development and remodelling can have significant consequences systemically. This may be due to the emerging role of WAT as a key endocrine organ, responsible for the production of metabolic regulatory hormones such as leptin and adiponectin (165).

In contrast to WAT, brown adipose tissue (BAT) is a very metabolically active tissue that plays a role in thermoregulation. BAT has a large capacity for glucose uptake and fatty acid oxidation and has significant expression of uncoupling protein (UCP)-1, a unique protein able to dissipate the electrochemical gradient across the inner mitochondrial membrane resulting in a direct conversion of chemical energy to heat (26). However, due to the low abundance of BAT found in humans, (estimates range between 60 and 100 grams), the contribution of BAT to whole body glucose homeostasis remains low, at least when not activated by cold exposure (93).

Diets used in obesity/diabetes research

The high fat diet

Altering rodent diets to produce and/or exacerbate obesity and metabolic syndrome has been a common tool used by researchers dating back to the mid 20th century. Early studies promoted HFD as a way to induce obesity, hyperglycaemia and insulin resistance and this type of diet has subsequently become a widely used tool in modelling the metabolic syndrome (59, 88, 144, 168). Although extensively used in diabetes and obesity research, the composition and fat content of "so called" HFD differ considerably, with relative fat fractions varying from between 20-95%, with fat derived from multiple sources including animals (lard, tallow), plants (olive, safflower, corn, coconut) and fish (21). Although HFD studies in rodents generally constrain the amount of dietary fat to between 45-60% of energy intake, there is an increasing field of literature looking at the effect of very high fat diets (VHFD) (80-95% dietary energy as fat) on metabolic health similar to low carbohydrate, ketogenic diets in humans (71). In rodents the evidence indicates that although VHFD generally

241 seem to reduce body weight gain (although not always (91)), they also reduce
242 systemic and particularly hepatic insulin sensitivity (10, 79, 91)
243
244 Because the source of fat used in HFD can vary, it is important to keep in mind that
245 feeding rodents diverse fatty acid species has been demonstrated to lead to different
246 metabolic outcomes. This is outlined in Table 1, a review of studies investigating
247 insulin sensitivity by hyperinsulinemic-euglycemic clamp of rodents fed varying diets
248 (high fat and refined carbohydrate). In rats, diets high in saturated fatty acids (SFA)
249 have been shown to cause a greater degree of insulin resistance than diets high in
250 mono (MUFA) and polyunsaturated fats (PUFA) (20, 158). Different SFA subtypes
251 may also differ in their effects on insulin action with some evidence showing a greater
252 effect of stearate (181). There is also evidence from rodents fed a mixed fatty acid
253 diet that different fatty acid species may have tissue specific effects on insulin action
254 (190). In mice, diets containing predominantly PUFA reportedly produce lower levels
255 of muscle TAG and DAG than ones with higher levels of SFA and MUFA (172),
256 however this does not necessarily translate to a difference in insulin sensitivity (125).
257 Interestingly, supplementing HFD with omega-3 fatty acids seems to improve insulin
258 sensitivity and muscle insulin action (97, 128, 158, 159), possibly through a PPARα
259 dependent mechanism (112). Additionally, a HFD consisting predominantly of
260 medium chain SFAs (MCFA) such as lauric and capric acid (the predominant fatty
261 acid species in coconut oil) do not seem to reduce insulin sensitivity as much as a
262 HFD containing animal derived long chain SFAs (20, 164, 178, 188). This may be
263 due to contrasting effects of these FAs on insulin sensitive tissues such as skeletal
264 muscle (107) and liver (164). This is of particular interest because hydrogenated
265 coconut oil has been commonly used as a saturated fatty acid source for HFD and is

found in the widely used, commercially available obesogenic “Surwit diet” (166, 167) (Research Diets D12330, D12331). However other researchers have reported no difference in insulin sensitivity between mice fed a HFD predominantly of either MCFA or long chain SFAs (LCFA) (41).

The mechanisms behind the differential effects of saturated and unsaturated fatty acids on insulin sensitivity are still debated and include alterations in plasma membrane phospholipid composition (122, 158, 187), transcriptional changes in fatty acid metabolism (35), inflammatory effects (151) as well as direct regulation of insulin signalling (33, 65, 107, 146, 161, 193). It is also clear that the rate of oxidation of different species of fatty acids vary quite significantly and fatty acids that are more unsaturated and shorter seem to be oxidized faster than longer, more saturated fatty acids (46, 95). This may be relevant as incomplete fatty acid oxidation and substrate competition between fatty acids and glucose (glucose/fatty acid cycle) have both been implicated as possible mechanisms for reduced insulin-stimulated glucose uptake in peripheral tissue (109, 134, 177).

<u>High fat, high sucrose diet</u>

In the late 1980s Surwit *et al* published a diet induced obesity study on C57BL/6J using a high fat, high sucrose diet (HFHS). The authors concluded that the C57BL/6J strain of mice is genetically predisposed to DIO and that a mix of a high fat, high simple carbohydrate diet is sufficient to produce many of the pathophysiological changes of the metabolic syndrome (167). In a follow up study, they showed that a HFHS diet generates higher weight gain and more pronounced hyperglycaemia and hyperinsulinemia than a high fat, low carbohydrate diet (166). These two papers

291 popularised the HFHS diet as a way of generating a more severe metabolic syndrome
292 phenotype in the C57BL/6J model of DIO. The HFHS diet is widely used in rodent
293 studies as a "western style" diet due to high levels of both sucrose and fat and is
294 thought to more closely resemble a high energy human diet rather than high and very
295 high fat diets of 60% or greater calories from fat. There is currently limited data on
296 direct comparisons between the more traditional HFD and the Surwit HFHS diets
297 effects on insulin action although Omar *et al* have reported that in C56BL6/J mice,
298 the HFHS is not as effective as a more traditional HFD in supressing whole body
299 insulin action under hyperinsulinemic-euglycemic clamp conditions (116).
300
301 Cafeteria and "choice" diets
302 The use of a non-purified "cafeteria" diet, in which a mix of energy dense high fat and
303 high sugar foods that are regularly consumed by humans (cake, biscuits, chips and
304 processed meats) has been gaining traction as a more physiologically relevant to
305 model the human western diet. The cafeteria diet has been reported to generate more
306 pronounced obesity than a regular high fat diet (63, 140) and adding a greater degree
307 of food choices seems to increase food intake and change feeding behaviour (104).
308 Recently, Diepenbroek *et al* investigated the effect of different choice diets (animals
309 received access to a combination of chow, beef tallow and 30% sucrose solution) on
310 insulin sensitivity in rats after 1 week of diet. They found that animals who only had
311 access to chow and tallow had the greatest reduction in hepatic glucose production
312 while rats with access to all three foods were substantially hyperphagic and had the
313 lowest whole body glucose disposal (48). Due to the current lack of studies using
314 cafeteria and choice diets, no major conclusion can be drawn about the comparative

effects of a more conventional HFD and these diets on insulin action. Additionally, the cafeteria diet may make energy intake measurements harder to perform.

High carbohydrate diets

Although not as widely used as HFD in rodent DIO models, diets high in refined simple carbohydrates have been used successfully to model some elements of the metabolic syndrome, in particular hypertriglyceridemia and insulin resistance. Sprague Dawley and Wistar rats have been shown to become insulin resistance after 4-8 weeks of either high sucrose or fructose diets (60-70% sucrose or fructose as a percentage of total calories) (39, 119, 121, 145, 160). Pagliassotti *et al* reported that in as little as one week of high sucrose feeding, Wistar rats show evidence of whole body insulin resistance driven by reduced hepatic glucose suppression (120). There are fewer studies investigating systemic insulin resistance in mice fed diets high in simple carbohydrates although high sucrose/fructose feeding has been shown to reduce glucose tolerance compared to mice fed a diet high in complex carbohydrate (105, 135, 139, 162). However others have reported no difference in glucose disposal under hyperinsulinemic-euglycemic clamp conditions (116).

Although the induction of whole body insulin resistance by high sucrose/fructose feeding is not as well established in rodents as the HFD model of insulin resistance, it is clear that other hallmarks of the metabolic syndrome such as obesity, hypertriglyceridemia and NAFLD, all of which correlate with insulin resistance in rodents and humans, can be quite pronounced (6, 127). Interestingly, the fructose moiety of the sucrose molecule seems to be the main driver of the loss in insulin

339 action possibly because of its preferential metabolism in the liver and the subsequent
340 increase in liver lipids as a result (6, 170, 171). The molecular mechanisms behind
341 insulin resistance caused by dietary fructose are mainly focussed on the strong
342 hypertriglyceridemia and hepatic lipid accumulation explained in detail by Basciano
343 *et al* (6). Additionally, high sucrose/fructose feeding may alter the trafficking
344 dynamics of glucose transporters (174) and potentially trigger ER stress pathways
345 (135). It is unclear whether insulin resistance derived from high simple carbohydrate
346 feeding is of a similar aetiology to that derived from high fat diets. Potentially, a
347 chronic increase in insulin secretion or glucose oversupply may drive the insulin
348 resistance similar to insulin resistance seen in glucose-infused rodents (14, 68). From
349 the few comparative studies published it seems likely that in rodents, high fat and
350 high fat/high sucrose diets generate a greater degree of insulin resistance than high
351 sucrose/fructose diets (116) (See Table 1).

352 Altering the source or complexity of more complex carbohydrates can also have
353 implications on whole body insulin sensitivity. Replacing regular starch with resistant
354 starch reduces digestion in the small intestine and increases bacterial fermentation in
355 the large intestine. Resistant starch is a modified starch that generally contains higher
356 levels of amylose (one of the two main components of starch, the other being
357 amylopectin) or more cross-linkages within the starch structure. Rats fed a high
358 carbohydrate diet higher in resistant starches exhibited less weight gain and better
359 insulin sensitivity than those on a simple starch diet (24, 64, 150).
360
361 <u>Altering dietary protein</u>
362 Historically, protein is generally kept consistent in rodent DIO models at
363 approximately 20% of the dietary energy and therefore there are relatively few studies

that investigate insulin action where dietary protein has been altered. Rossetti *et al* found that male Sprague-Dawley rats were significantly insulin resistant when fed very high protein diets (calorically 63.2% and 36.5% protein) due to both a decrease in peripheral glucose uptake and increased HGP (138). More recently, Solon-Biet *et al* explored the metabolic effects of varying dietary protein and showed that diets that were low in protein and high in carbohydrate were associated with improved glucose tolerance in mice despite the high-protein fed mice being leaner (153). This investigation also indicated that, when faced with low levels of dietary protein, rodents compensate by overconsumption to reach their protein target. This has implications when comparing diets that differ greatly in protein content.

The source of protein in synthetic diets has also been shown to influence insulin action. Animals fed fish protein have been shown to have enhanced systemic insulin sensitivity compared to those fed primarily casein or soy (92) and protein from specific fish species such as salmon may particularly promote insulin sensitivity (129). Others have shown that in combination with a high sucrose diet, replacing the source of dietary protein from casein to soy has beneficial effects on insulin resistance and dyslipidaemia (115). The reasons why proteins from different sources may have differential effects on insulin sensitivity remain poorly understood. Early work suggested a link with GLUT4 translocation in muscle (92). This may be due to the differential effect of specific amino acids on the amino acid sensing kinases, mTOR and S6 Kinase that have been shown to interact with the canonical insulin signalling cascade (31, 180, 192). There is also emerging evidence that circulating levels of branched chain amino acids (BCAA) correlate with impaired insulin signalling and action (75, 100, 113). Newgard *et al*, found that rats fed a HFD supplemented with

BCAA had similar glucose tolerance despite decreased adiposity compared to fat fed controls (113) however evidence of the effect of dietary BCAA on insulin action in rodents is limited.

General considerations in planning a rodent diet study

Length of Diets

The length of time mice are exposed to different diets for diet-induced obesity studies varies considerably with common DIO models being fed for between 4 to 16 weeks in order to elicit observable differences in body and fat mass. The different pathologies of the metabolic syndrome occur at different times in this process with insulin resistance reported to occur quite rapidly between 1-3 weeks after commencing a HFD. After one week of a HFD, hepatic insulin resistance is detectable through the lack of suppression of hepatic glucose output in a hyperinsulinemic-euglycemic clamp, while skeletal muscle insulin resistance was not evident until 3 weeks after commencing the diet (86, 124, 179). Visible differences in macrophage "crown-like" structure numbers in adipose tissue take longer to appear, and become visible around 5 weeks of HFD and continue to increase in number at 10 and 16 weeks (94, 179). Adipose tissue inflammation may be responsible for further loss of insulin action after long term fat feeding (94), however is unlikely to be responsible for early lipid-induced insulin resistance due to this timing discrepancy. Interestingly, multiple investigations have found that systemic insulin resistance in C56BL6/J mice as measured by glucose infusion rate (GIR) during a hyperinsulinemic-euglycemic clamp decreases with fat feeding without further deterioration after 3 weeks (124, 179). Pagliassotti *et al* found a similar result in high sucrose fed rats where there was

no further deterioration of systemic insulin sensitivity after 5 weeks of high sucrose feeding (120).

Although, a loss of insulin action can take weeks to be observed, there is some evidence suggesting that a proportion of lipid-induced insulin resistance can be reversed by relatively short periods of carbohydrate administration. This can be seen in fat-fed rats given a high glucose meal the day before a hyperinsulinemic-euglycemic clamp (7, 114). A similar "glucose priming" effect was seen in dogs administered a duodenal infusion of glucose the morning before a clamp (108). However others have suggested that this insulin resistance is an adaptive response to high serum free fatty acid levels due to a functional glucose/fatty acid cycle and that muscle insulin resistance caused by fat-feeding cannot be reversed in the same way (60). Similarly, mice fed a HFD for 8 weeks returned to normal glucose tolerance after a 7-day switch to chow despite having similar adiposity to HFD controls (85).

Strain comparisons

When using rodent DIO models it is not uncommon to extrapolate that the metabolic effects of a certain diet are relevant to all strains of mice. However diverse strains of rodents have been shown to have different metabolic profiles and adapt in different ways to nutrient excess. For example, C57BL/6 mice are the strain used most in metabolism research however, this strain exhibits smaller reduction in insulin sensitivity (measured by clamp) upon exposure to a HFD compared to the DBA strain (8). BALB/c mice, another common strain, seem to resist the effects of a HFD on glucose tolerance (106). There is also some evidence to suggest that there is a

437 difference in glucose handling between Wistar and Sprague-Dawley strains of lab rat
438 (102).
439

440 Gender

441 The majority of studies looking at dietary manipulation and metabolism are conducted
442 in male mice. This is due in part to concerns about what impact the oestrus cycle
443 might be having on metabolic parameters in females independent of the dietary
444 manipulation, and because of the reported protective effect of female hormones on
445 metabolism (25, 117, 136). It has been demonstrated that high fat or high sucrose
446 feeding in female "pre-menopausal" rats does not produce the same glucose
447 intolerance or insulin resistance as in male rats (3, 67). Increasing plasma free fatty
448 acids using an intralipid infusion also has been shown to cause a decrease in insulin
449 sensitivity in male but not female rats (62). This was suggested to be related to the
450 fact that females have better oxidative stress responses (11, 23, 29), have more
451 mitochondria (3, 80), with better respiratory capacity (80, 110) or that they have a
452 greater capacity to expand fat stores (3). Whatever the reason, this protective effect is
453 lost by removal of the ovaries (136).
454

455 Palatability of diets

456 An important consideration when undertaking diet studies is the palatability of the
457 diets. This is especially the case when comparing diets that have different consistency
458 or smell like starch-based diets with high fat diets. A significant amount of evidence
459 shows that different carbohydrate and fat formulations are differentially palatable in
460 rodents (147). High carbohydrate diets that are in a liquid or gel form produce more

461 substantial hyperphagia and weight gain compared to pelleted or powdered dry diets
462 (51, 132, 133) and even powdering food has been reported to negate the body weight
463 changes observed between pelleted low-fat control diets and high-fat and western
464 diets (47). In the context of a particular diet promoting fat gain to allow examination
465 of the metabolic complications of obesity it is important to consider how difficult the
466 diet is to consume as this may not be dependent on the macronutrient content of the
467 particular diet.
468
469 Choice of the most suitable control diet is also important. It is very common for a
470 "chow" diet to be used as a low fat control diet as it is a cheaper alternative to a
471 matched semi-purified diet. However, the content of "chow" is often not well defined
472 and the sources of macronutrients used by different suppliers can vary due to
473 availability and cost. Warden *et al* discuss the problems of using chow control diets
474 (184). However, it is worth noting, as discussed above that semi-purified low fat
475 control diets, generally using starch as the carbohydrate source, can produce similar
476 levels of obesity as a high fat diet if the diet is powdered (47). Due to the different
477 physical properties of carbohydrates and lipids it is possible that in a dried and
478 pelleted form high fat diets are significantly more palatable than high starch diets.
479
480 Isocaloric diets
481 Due to the differences in energy density between carbohydrate and fats (16kJ/g
482 carbohydrate, 37kJ/g fat) (189) comparing HFD with low fat control diets will result
483 in a HFD that is more calorically dense than the control diet. For this reason, many
484 investigators normalize the energy per gram of the diet by the addition of non-
485 digestible ingredients such as cellulose or inulin. The benefits of this approach is that

it provides a more accurate way in which to measure food intake as well as reducing the difference in food volume eaten by feeding diets with different energy densities. However, the effect of large amounts of non-digestible fiber may have effects on the microbiome (32), satiety, gastric emptying and the incretin response (123) that must be taken into consideration when planning or analysing results from any dietary study. Altering the diet by the addition of fibre to increase short chain fatty acid production from gut fermentation has been shown to have beneficial effects on systemic insulin sensitivity (9). Another important key point in designing animal studies is that the diets meet the animal's minimal nutrient requirements, especially for protein, vitamins and minerals, to eliminate the possibility of overconsumption of the diet to fulfil needs for specific nutrients.

Thermoneutrality

Recently, there has been considerable discussion about the applicability to human diseases of studies using rodents housed at temperatures (19-24°C) which are more attuned to suiting the comfort of the human researchers than the comfort of the rodents. Mice expend a significant amount of energy on thermogenesis at temperatures of 19-24°C that can mask the development of metabolic and other phenotypes that might be central to the aims of the particular study (27). For example, in the case of uncoupling protein (UCP)-1 KO animals, a paradoxical phenotype that showed a resistance to high fat diet was observed at 20°C, but not at 27°C indicating the relevance of housing temperature to understand the role of UCP-1 in energy balance (98). In another example Castillo *et al* reported that mice with deletion of type 2 deiodinase (DIO2; enzyme in thyroid hormone metabolism) have no phenotype at 22°C but became more glucose intolerant and obese at 30°C on a high fat diet (28).

Therefore, many are advocating housing mice at “thermoneutral” temperatures (28°C-34°C) where they expend minimal energy on thermogenesis (99, 101). However, others have argued that mice with adequate nesting material that are group housed may have a much lower thermoneutral housing temperature approximating 20-22°C (154).

Hyperinsulinemic-euglycemic clamp procedure

The hyperinsulinemic-euglycemic clamp in rats (22, 87) and mice (182) was adapted from the human procedure (pioneered by DeFronzo *et al* (42)) and is considered the gold-standard for assessing insulin sensitivity *in vivo*. In the human procedure, insulin is often infused sequentially at two different rates (2-step clamp), first a lower rate in order to quantify the contribution of HGP, and then a higher rate in which it is assumed that HGP is completely supressed, to assess the contribution of the periphery. In comparison, the rodent model generally utilizes the infusion of radioactive glucose tracers to measure HGP (either by isotope dilution or disappearance of a tracer bolus) and therefore is generally conducted at only one rate of insulin infusion. However, it is worth considering that at high insulin doses, HGP may be completely supressed, therefore when trying to discern differences in hepatic insulin sensitivity it may be beneficial to utilize a lower insulin infusion rate (121, 152). Ayala *et al* provide an in-depth investigation into the considerations when designing a clamp study in mice (4, 5) including insulin doses, use of anaesthesia and site of blood sampling. Standard operating procedures for the hyperinsulinemic-euglycemic clamp in mice (5) and rats (70) have been described in detail. As with the other considerations outlined in this review, when designing clamp studies in rodents, some thought should be given to

whether the primary outcome required from the procedure is accurate assessment of hepatic or peripheral insulin sensitivity.

Conclusion

The heterogeneity of metabolic phenotypes reported in the literature for HFD models of DIO (outlined in Table 1) may be in part due to the disparity in percentage fat content (ranging from around 45% all the way up to 95%) and the specific source of fat. There continues to be no standardized definition of a “high fat diet” however it is quite clear that diets with a fat content greater than 40% promote systemic insulin resistance in all of the studies reviewed here (Table 1). These include both studies using chow and semi-purified diets as a control. There may be an inverse relation between the percentage of fat in the diet and insulin sensitivity however as the VHFDs generally have substantially reduced protein content it can be hard to make conclusion about the importance of the excess of one macronutrient over the lack of another. The evidence is less clear with the high sucrose/fructose diets, which seem to have a clear negative effect on hepatic insulin sensitivity but may not have as strong an effect on peripheral glucose disposal as a more traditional HFD. A summarised graphical depiction of the studies investigated in Table 1 comparing the peripheral and hepatic effects of varying diets on insulin sensitivity is illustrated in Figure 1.

Clearly, the molecular mechanisms that govern insulin sensitivity in humans are still not well understood. We believe that human research in this field is of vital importance however due to the difficulties and ethical problems that result in giving unhealthy diets to humans for long periods of time, it is still almost impossible to conduct well-controlled dietary studies in humans that require one group to gain

weight or impair metabolic health. This can result in particular research areas in which human data presents particularly conflicting results such as the effect of dietary sugar on insulin action. Although rodent experiments show clear detrimental effects of high dietary sucrose/fructose on insulin sensitivity, human studies have been much more mixed, potentially due to fears about leaving humans on unhealthy diets for long periods of time or due to rodents having a higher dietary sugar percentage (40). The use of rodents to model the metabolic effect of diets on humans remains controversial, with some groups seeing no translatable potential (90). However, rodents remain a useful tool to investigate the mechanisms that drive insulin resistance, which we know is an evolutionary conserved phenomenon present in mammals and some non-mammal vertebrates (58, 194). Rat and mice dietary models investigating insulin action are therefore still required as they have more relevance to human diet-induced metabolic syndrome than monogenic models of insulin resistance and provide important tools for the evaluation of novel therapeutic agents for insulin resistance and type 2 diabetes. However, the field is moving away from focussing solely on the idea that HFDs generate a generic and equivalent insulin resistance. An array of different diets which effect insulin action in both negative and positive ways are now being used including variations in protein type and content to create a new dimension in the macronutrient composition of diets (153). These on-going and future studies will provide a new perspective on the effects of diet on metabolic health in rodents, which may translate to better health outcomes in humans.

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Table 1 Rodent hyperinsulinemic-euglycemic clamp studies comparing the effects of dietary content.

Predominant Energy Source	Citation	Rodent Sex/Strain/Age or weight#	Diet (energy % of macronutrient)	Control /Comparative Diet	Length of Diet (days)	Muscle Glucose Uptake (% control)	Hepatic Glucose Production† (Δ % control)	Glucose Infusion Rate (% control)
Saturated Fat*	(158)	Male Wistar Rats (age 54 days)	Tallow (59% fat, 20% carb, 21% protein)	Standard chow (12% fat, 65% carb, 23% protein)	30	27 (RQ)	40	43
	(116)	Female C57BL6/J mice (age 6 weeks)	Lard (60% fat, 20% carb, 20% protein) (Research Diets #D12492)	Sucrose, starch. (10% fat, 70% carb {35% sucrose}, 20% protein) (research diet #D12450B)	56	NR	NR	55
	(56)	Male C57BL6/J mice (age 7-8 weeks)	Lard (60% fat, 20% carb, 20% protein) (Research Diets #D12492)	Standard chow (energy not given)	10	NR	31	55
	(41)	Male C57BL6/J mice (age 12 weeks)	Long-chain triacylglycerol (75% palmitate) (source not reported) (45% fat, 30% carb, 25% protein)	Standard chow (12% fat, 61% carb, 27% protein)	56	NR	31	40
	(79)	Male C57BL6/J mice (age 7 weeks)	Lard (95.1% fat, 0.4% carb, 4.5% protein) (F3666 Bio-Serv).	Standard chow (17% fat, 60% carb, 23% protein)	35	123 (Q)	28	44
	(10)	Male Wistar Rats (age 12 weeks)	Tallow (78.7% fat, 2.2% carb, 19.1% protein)	Starch (semipurified) 16.7% fat, 64.3% carb, 19% protein)	28	NR	84	40
	(10)	Male Wistar Rats (age 12 weeks)	Tallow (92.8% fat, 1.7% carb, 5.5% protein)	Starch (semipurified) 16.7% fat, 64.3% carb, 19% protein)	28	NR	115	20
Medium Chain Fatty Acids	(178)	Male Wistar Rats (age 8 weeks)	Hydrogenated coconut oil (59% fat, 20% carb, 21% protein)	Standard chow (12% fat, 65% carb, 23% protein)	35	90 (RQ)	13	82
	(188)	Male Wistar Rats (~280g start of study)	Low fat basal diet supplemented with octanoic-(70%)/decanoic (30%) acid.	Standard chow (energy not given)	28	NR	NR	90

Table 1 Rodent hyperinsulinemic-euglycemic clamp studies comparing the effects of dietary content. (continued)

Predominant Energy Source	Citation	Rodent Sex/Strain/Age or weight#	Diet (energy % of macronutrient)	Control /Comparative Diet	Length of Diet (days)	Muscle Glucose Uptake (% control)	Hepatic Glucose Production† (Δ % control)	Glucose Infusion Rate (% control)
	(116)	Female C57BL6/J mice (age 6 weeks)	Hydrogenated coconut oil (58% fat, 25.5% carb {13% sucrose), 16.4% protein)(Research Diets #D12331).	Sucrose, starch. (10% fat, 70% carb {35% sucrose}, 20% protein) (Research Diets #D12450B)	56	NR	NR	78
	(41)	Male C57BL6/J mice (age 12 weeks)	Medium-chain triacylglycerol (source not reported) (45% fat, 30% carb, 25% protein)	Standard chow (12% fat, 61% carb, 27% protein)	56	NR	18	41
High Fat, High Sucrose (Surwit)	(181)	Male C57BL6/J mice (age 14 weeks)	Lard (45% fat, 35% carb {17.5% sucrose}, 20% protein) (Research Diets #D12451).	Sucrose, starch. (10% fat, 70% carb {35% sucrose}, 20% protein) (Research Diets #D12450B)	35	NR	40 (Difference in suppression)	28
	(179)	Male C57BL6/J mice (age 8-12 weeks)	Cocoa butter (43% fat, 40% carb {32% sucrose}, 17% protein) (Specialty feeds # SF01-028)	Standard chow (5% fat, 72% carb, 23% protein)	7, 21, 42, 112	112, 64, 48, 54 (Q)	23, 38, 39, 38	69, 42, 44, 38
Monounsaturated Fat	(158)	Male Wistar Rats (age 54 days)	Olive oil (59% fat, 20% carb, 21% protein)	Standard chow (12% fat, 65% carb, 23% protein)	30	68 (RQ)	14	63
Polyunsaturated Fat	(88)	Male Wistar Rats (age 90-120 days)	Safflower oil (60% fat, 20% carb, 20% protein)	Standard chow (12% fat, 65% carb, 23% protein)	22	57 (RQ)	NR	66
	(159)	Male Wistar Rats (age 60 days)	Safflower oil (59% fat, 20% carb, 21% protein)	Standard chow (12% fat, 65% carb, 23% protein)	29	58 (RG)	34	48
	(158)	Male Wistar Rats (age 54 days)	Safflower oil (59% fat, 20% carb, 21% protein)	Standard chow (12% fat, 65% carb, 23% protein)	30	38 (RQ)	22	50
	(86)	Male Wistar Rats (300-380g final weight)	Safflower oil (59% fat, 20% carb, 21% protein)	Cornstarch (10% fat, 69% carb, 21% protein)	3, 21	79 (RQ) (3 day only)	43, 37	41, 54
	(30)	Male Wistar Rats (age 2 months)	Safflower oil (59% fat, 20% carb, 21% protein)	Standard chow (10% fat, 65% carb, 25% protein)	300	49 (RQ)	45	32
	(178)	Male Wistar Rats (age 8 weeks)	Safflower oil (59% fat, 20% carb, 21% protein)	Standard chow (12% fat, 65% carb, 23% protein)	35	57 (RQ)	21	56

Table 1 Rodent hyperinsulinemic-euglycemic clamp studies comparing the effects of dietary content. (continued)

Predominant Energy Source	Citation	Rodent Sex/Strain/Age or weight#	Diet (energy % of macronutrient)	Control /Comparative Diet	Length of Diet (days)	Muscle Glucose Uptake (% control)	Hepatic Glucose Production† (Δ % control)	Glucose Infusion Rate (% control)
	(119)	Male Sprague-Dawley rats (age 3, 8, 16, 56 weeks)	Corn oil (45% fat, 35% carb, 20% protein)	Cornstarch (12% fat, 68% carb, 20% protein)	35	57, 60, 50, 117 (S)	NR	R_d (72, 77, 75, 90)
	(112)	Male Sv/129 mice (age 10-12 weeks)	Safflower oil (59% fat, carb, protein not reported)	Standard chow (7% fat, carb, protein not reported)	14	116 (Q)	69	48
Marine Oils	(158)	Male Wistar Rats (age 54 days)	Safflower oil, fish oil (59% fat {18% fish oil}, 20% carb, 21% protein)	Standard chow (12% fat, 65% carb, 23% protein)	30	95 (RQ)	-3	94
	(112)	Male Sv/129 mice (age 10-12 weeks)	Safflower oil, fish oil (59% fat {22% fish oil}, carb, protein not reported)	Standard chow (7% fat, carb, protein not reported)	14	150 (Q)	24	75
	(159)	Male Wistar Rats (age 60 days)	Safflower oil, fish oil (59% fat {12% tuna oil}, 20% carb, 21% protein)	Standard chow (12% fat, 65% carb, 23% protein)	29	100 (RG)	5	94
Fructose/Sucrose	(83)	Male Wistar Rats (age 6 weeks)	Sucrose (10% fat, 63% carb {63% sucrose}, 27% protein)	Standard chow (13% fat, 61% carb, 26% protein)	28	NR	11	105
	(170)	Male Wistar rats (260g)	Fructose (10% fat, 69% carb {34.5% fructose}, 21% protein.	Glucose (10% fat, 69% carb {34.5% glucose}, 21% protein.	30	50 (RQ)	NR	63
	(116)	Female C57BL6/J mice (age 6 weeks)	Sucrose (11% fat, 73% carb {60% sucrose} 16.4% protein) (Research Diets #D12329)	Sucrose, starch. (10% fat, 70% carb {35% sucrose}, 20% protein) (Research Diets #D12450B)	56	NR	NR	105
	(121)	Male Wistar rats (180g)	Sucrose (12% fat, 68% carb {68% sucrose}, 20% protein)	Cornstarch (12% fat, 68% carb, 20% protein)	56	NR	63	48
	(120)	Male Wistar rats (200-220g)	Sucrose (12% fat, 68% carb {68% sucrose}, 20% protein)	Cornstarch (12% fat, 68% carb, 20% protein)	7, 14, 35, 56	NR	38, 65, 68, 71	60, 47, 40, 40
	(119)	Male Sprague-Dawley rats (age 3, 8, 16, 56 weeks)	Sucrose (12% fat, 68% carb {68% sucrose}, 20% protein)	Cornstarch (12% fat, 68% carb, 20% protein)	35	100, 74, 57, 91 (S)	NR	R_d (97, 84, 78, 87)

Table 1 Rodent hyperinsulinemic-euglycemic clamp studies comparing the effects of dietary content. (continued)

Predominant Energy Source	Citation	Rodent Sex/Strain/Age or weight#	Diet (energy % of macronutrient)	Control /Comparative Diet	Length of Diet (days)	Muscle Glucose Uptake (% control)	Hepatic Glucose Production† (Δ % control)	Glucose Infusion Rate (% control)
	(39)	Male Sprague-Dawley rats (age 12 weeks)	Fructose (12% fat, 66% carb {66% fructose}, 22% protein)	Standard chow (4% fat, 72% carb, 24% protein)	56	NR	NR	55

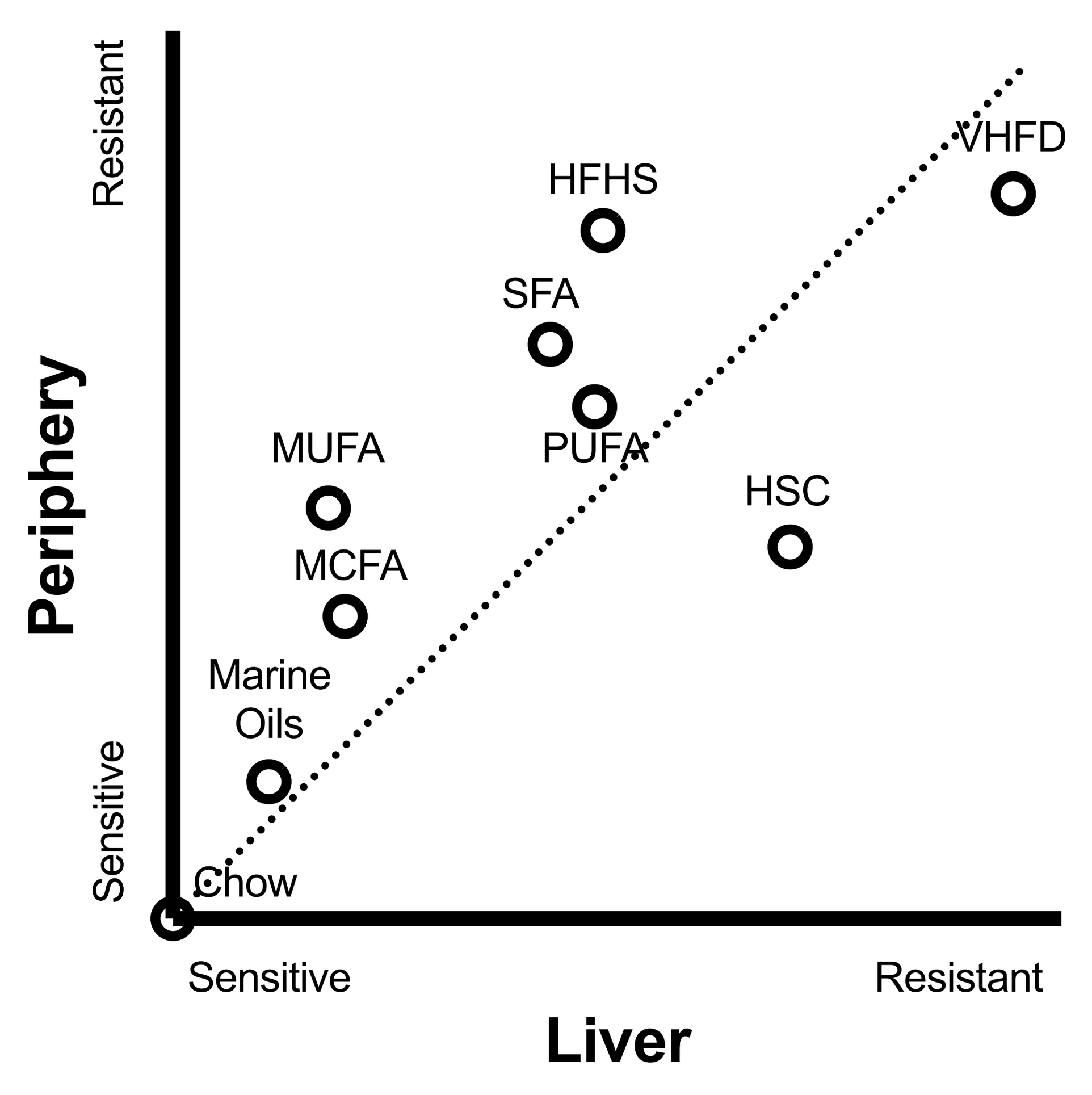
The studies in this table were identified by searching for "hyperinsulinemic-euglycemic clamp in mice/rats" in addition to each dietary subtype (for example "high sucrose/fructose diet"). Studies focussing on transgenic rodent lines or pharmaceutical intervention were generally excluded due to a primary focus on comparisons between genotype or drug. Additionally, due to many of the studies controlling for energy intake either through pair feeding or isocaloric diets, we have not included body weight gain. Reporting insulin levels during the clamp was not an exclusion criteria as many of the studies were performed before this became commonplace. There is significant heterogeneity between clamp methodologies between groups, including rates of insulin infusion, anaesthesia, fasting time, bleeding from the tail, vein or artery and glucose tracers used. These may all have effects on the rates gained from the experiment as described by Ayala *et al* (4, 5). Consequently, glucose metabolic rates are shown as per cent of the control diet as the actual rates show significant disparity, making comparison between different studies more difficult. In studies where data was presented in graphs and exact numbers were not reported, percentages were estimated from the graphs displayed and may vary slightly from the results obtained. R_d – Rate of glucose disappearance. RQ – Red quadriceps. Q – Quadriceps. RG – Red gastrocnemius. S – Soleus. NR – Not reported.

Age and weight at the start of study unless specified.

* Although lard and tallow contain high levels of saturated fat (38-50%), they can be higher in monounsaturated fatty acids. However due to relatively high level of saturated fat compared to other fatty acid sources (safflower oil, olive oil, corn oil), they have been grouped with saturated fats.

† As many of the control groups had completely supressed hepatic glucose production (HGP) ($R_a = 0$) it was not possible to express HGP as simply % control. Therefore, we calculated the difference in HGP between groups as a function of R_d in the following equation ((control diet GIR/R_d) - (test diet GIR/R_d)) *100). A higher number indicates that the test diet has a higher rate of HGP as a percentage of R_d.

Figure 1 **A comparison of peripheral and liver insulin sensitivity between different diets.** This graph is a visual depiction of the HGP and GIR values from Table 1, averaged between studies using the same broad diet classifications. The x and y axes rank diets based on their average HGP (liver) and GIR (periphery) from insulin sensitive to insulin resistant. As the HGP is a function of GIR and R_d, this graph is not meant to describe whether a specific diet produces greater insulin resistance in the liver compared to the periphery, rather it is a comparison between different diets. The data depicted is an average between different investigations with varying dietary makeups, rodent strains, length of diets and many other differences and should not be taken as a true comparative study (of which several exist in the literature (116, 158)). VHFD – very high fat diet. HFHS – high fat high sucrose. SFA – saturated fatty acids. PUFA – polyunsaturated fatty acids. MUFA – monounsaturated fatty acids. HSC – high simple carbohydrate (sucrose/fructose). MCFA – medium chain fatty acids.


Resistant
Periphery
Sensitive
VHFD
HFHS
SFA
PUFA
MUFA
HSC
MCFA
Marine
Oils
Chow
Sensitive
Resistant
Liver