MOLECULAR METABOLISM

Commentary

Testing the carbohydrate insulin model in mice: Erroneous critique does not alter previous conclusion

The carbohydrate-insulin model (CIM) is a theoretical model which aims to explain the role of carbohydrates in driving adiposity, and hence how changing patterns of macronutrient intake have shaped the obesity epidemic [1,2]. The idea in the CIM is that ingestion of some types of carbohydrate leads to excessive release of insulin. This insulin causes the circulating glucose derived from the ingested carbohydrate to be efficiently taken up by tissues and converted into fat. In addition, suppression of adipose tissue lipolysis and stimulation of uptake of free-fatty acids lead to a state similar to starvation, which drives down energy expenditure (via an unspecified mechanism and in unspecified elements of expenditure) and also stimulates hunger. This hunger leads to higher intake in a vicious cycle resulting in positive energy balance. An important aspect of the model is that elevated food intake and low metabolism are considered to be a consequence of increasing adipose tissue fat content rather than a cause [2]. The CIM has also been invoked as an explanation for why diets that incorporate very low carbohydrate levels (so-called low carbohydrate-high fat LCHF or ketogenic diets) are successful in managing hunger and weight loss [1]. The carbohydrates that are perceived to be particularly problematical are those that lead to a rapid rise in circulating glucose levels that stimulate a large insulin release (high glycaemic index [GI] carbohydrates) [3].

A valuable aspect of the CIM is that it makes a number of clearly testable predictions. We recently tested these predictions [4] by exposing mice to a matrix of different diets which varied in their macronutrient compositions. We found that the predictions of the model proved to be largely incorrect. Hence, despite post-prandial insulin and fasting glucose levels following the model predictions, and insulin levels being correlated with inhibited lipolysis, the bottom line was that elevated carbohydrate in the diet did not lead to stimulated hunger, greater food intake, reduced energy expenditure or elevated adiposity. In their attached commentary on our paper, Ludwig et al. [5] suggest our work was not a 'meaningful test of the CIM' [5]. Their argument has several strands but is centred around details of the precise make-up of the diets we used. In particular they suggest a) the diets we used contain abnormal levels of macronutrients that would rarely be consumed by humans or wild rodents, b) the low carbohydrate diets were biased to include high levels of high GI carbohydrates (sucrose and maltodextrin), while the high carbohydrate diets were predominantly low GI carbohydrate (corn starch), c) our diets contained high levels of saturated fat which causes insulin resistance, which confounds any detection of an effect of the carbohydrate on insulin release. Finally, d) they direct attention to their own previous studies, referred to as '*appropriately designed rodent research*', where rats were exposed to high glycaemic index diets that produced data, including reduced energy expenditure, increased hunger and elevated adiposity, consistent with the CIM.

In this response we will address each of these comments in turn.

a) Unnatural dietary composition

In contrast to the suggestion that the diets we used would be rarely consumed by humans, the diets we used in our studies [4,6] included an extremely wide range of compositions — from 10 to 80% fat, 10—80% carbohydrate and from 5 to 30% protein. Some of these diets match very closely the macronutrient composition of the modern westernised diet with 15% calories from protein, 35% calories from fat and 50% from carbohydrates, while the other diets encompass most of the large individual variation in dietary selection among humans. Moreover, not only were the gross macronutrient compositions matched to the modern human diet, but the sub-components were also formulated to closely mimic the composition of the standard American diet, in terms of the saturated:mono-unsaturated:poly-unsaturated fat ratios, and the n-6:n-3 ratio (for details, see Hu et al. [6]). The diets also predominantly contained highly processed carbohydrates (corn starch, maltodextrin and sucrose) similar to those commonly consumed in the standard American diet. The statement that these diets would rarely be consumed by humans is therefore completely wrong. There are in fact very few human diets that our matrix does not cover.

Wild rodents also show a broad spectrum of dietary intake depending on food availability. For example, our own work on wood mice (*Apodemus sylvaticus*) showed that wild mice living at different sites only 15 km apart had radically different diets [7]. Wild house mice and rats also show extremely broad dietary intake patterns. It seems unlikely therefore that, given the very broad diet space covered by the dietary matrix we used, and the similarly wide diet choice of wild rodents, that these do not overlap. Consequently, the statement that these diets are unnatural is also unfounded.

b) Glycaemic index of the diets varied inversely with the level of carbohydrate

Ludwig et al. [5] separated the carbohydrates in our diets into high GI components (sucrose and maltodextrin) and low GI components (corn starch). They then pointed out that the high GI elements were over-represented in the diets with the lowest carbohydrate contents and visa-versa. They suggested that this compromises the design because the CIM refers only to high GI carbohydrates. This description of the diets by Ludwig et al. [5] is correct because we kept the sucrose and maltodextrin levels almost constant, and primarily varied the corn starch component. Hence, the diets with lower carbohydrate contents had higher proportions of sucrose and maltodextrin, and the higher carbohydrate content diets had higher proportions of corn starch. There is a problem, however, because their characterisation of sucrose and maltodextrin as high GI, and raw corn starch as low GI is completely wrong for C57BL/6 mice. The GIs of glucose, maltodextrin and cooked and raw corn starch in these mice are almost identical, when they are included as part of a diet containing 60% carbohydrate and fixed protein and fat contents [8], while the GI of sucrose in this dietary context is actually significantly lower. Because all our diets contained a fixed 5% sucrose, there was a slight bias for the low

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carbohydrate diets to also have slightly lower GI (the opposite of that highlighted by Ludwig et al. [5]). Hence, the suggested confound between carbohydrate level and carbohydrate GI is incorrect. Moreover, a more important index of glycaemic exposure is the glycaemic load rather than the glycaemic index. Glycaemic load is equal to the GI multiplied by the dose of carbohydrate, and this was certainly higher on the high carbohydrate diets than the low carbohydrate ones.

c) Insulin resistance confounds detection of carbohydrate effects

The diets we used did not have 'excessive' saturated fat levels (see above). Nevertheless, we agree that the mice on the high fat (and hence low carbohydrate) diets in our study were likely insulin resistant. However, this insulin resistance was not directly driven by the diet, but indirectly by the effect of the high fat diet on body fatness. Ludwig et al. [5] assert in the absence of any information that our mice had neuroinflammation, yet our RNAseq work in the hypothalamus did not indicate such. In effect, the argument by Ludwig et al. [5] comes down to, you cannot test the CIM in a situation where the mice ate a diet with high fat levels because high fat causes obesity and that leads to insulin resistance. Yet the whole point of the CIM is that it is not high fat that causes obesity, but high levels of high GI carbohydrates. Their argument in this context disproves their own theory. Moreover, we had two series of diets where fat levels were held constant and carbohydrate was traded off against protein, and in these situations increasing carbohydrate levels also did not precipitate elevated adiposity.

d) Previous 'appropriately designed' rodent studies support the CIM

Ludwig et al. [5] point to, and show a figure from, a previous study [9] that they indicate is more appropriate to test the CIM. This paper involved exposing rats to high GI (amylopectin) and low GI (amylose) carbohydrates in otherwise identical diets. The rats exposed to high GI carbohydrate had greater fat gain as predicted by the CIM. However, while Ludwig et al. [5] claim this paper also demonstrates lower energy expenditure and greater hunger in the high GI rats, there are no actual measurements of energy expenditure or hunger in the paper. Additionally, it is important to note that protracted feeding on these diets also induces insulin resistance [10], hence their argument that you cannot test the CIM in animals that have insulin resistance must surely also apply to their own previous study. Furthermore, an interesting aspect of the study was that the rats were surgically manipulated to remove 60% of their pancreas before the dietary manipulation started. The rationale for this procedure is unclear. However, it has a profound effect on the outcome because if this is not done, there is a different effect of GI on the body fatness [10]. Indeed, in this other prior study where rats were not surgically manipulated, the rats on the diet with highest GI carbohydrate intake gained the least body weight.
Overall, the comments by Ludwig et al. [5] on our study are completely unfounded. We continue therefore to assert that our data show that the carbohydrate-insulin model does not explain the impacts of different macronutrients on the body weight and adiposity of mice. The jury remains out on whether this refutation of the model is unique to mice or also pertains to humans.

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CONFLICT OF INTEREST

None declared.

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