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What We Know, Think We Know, or Are Starting to Know

We know that the body has very tightly regulated mechanisms to maintain glucose homeostasis. When these mechanisms start to break down, impaired glucose tolerance becomes the departure point that potentially ends up at a diagnosis of metabolic disease.

If we think about the mechanisms that regulate blood glucose levels, most of them are designed to protect against *too little* glucose in the blood, which likely would have been a more common occurrence during human evolution [(1)]. Several hormones – glucagon, adrenaline, cortisol, and growth hormone – may all act to *increase* blood glucose levels from low ranges and restore glucose homeostasis, but only insulin acts to decrease blood glucose levels from high ranges [(2,3)].

Food intake is the primary reason why blood glucose increases into higher ranges in what is termed the "postprandial period", i.e., the period after eating. Postprandial glycaemia*, the elevations in blood glucose which occur after a meal, may be influenced by numerous factors, including exercise and physical activity, time of day, time from the previous meal, and composition of the meal [(3)].

From the perspective of glucose tolerance, while carbohydrates take an obvious focus as the primary source of glucose in the diet, dietary protein has also attracted interest as it may influence both insulin secretion and, potentially as a result, lower postprandial glucose levels [(4,5)]. Dietary fat composition has also been shown to have a role in glucose tolerance, with saturated fats associated with impaired glucose and unsaturated fats associated with enhanced glucose tolerance [(6,7)].

With these factors, let' s get to the present study, which compared a high-protein diet to a Mediterranean diet in *female* participants with insulin resistance.

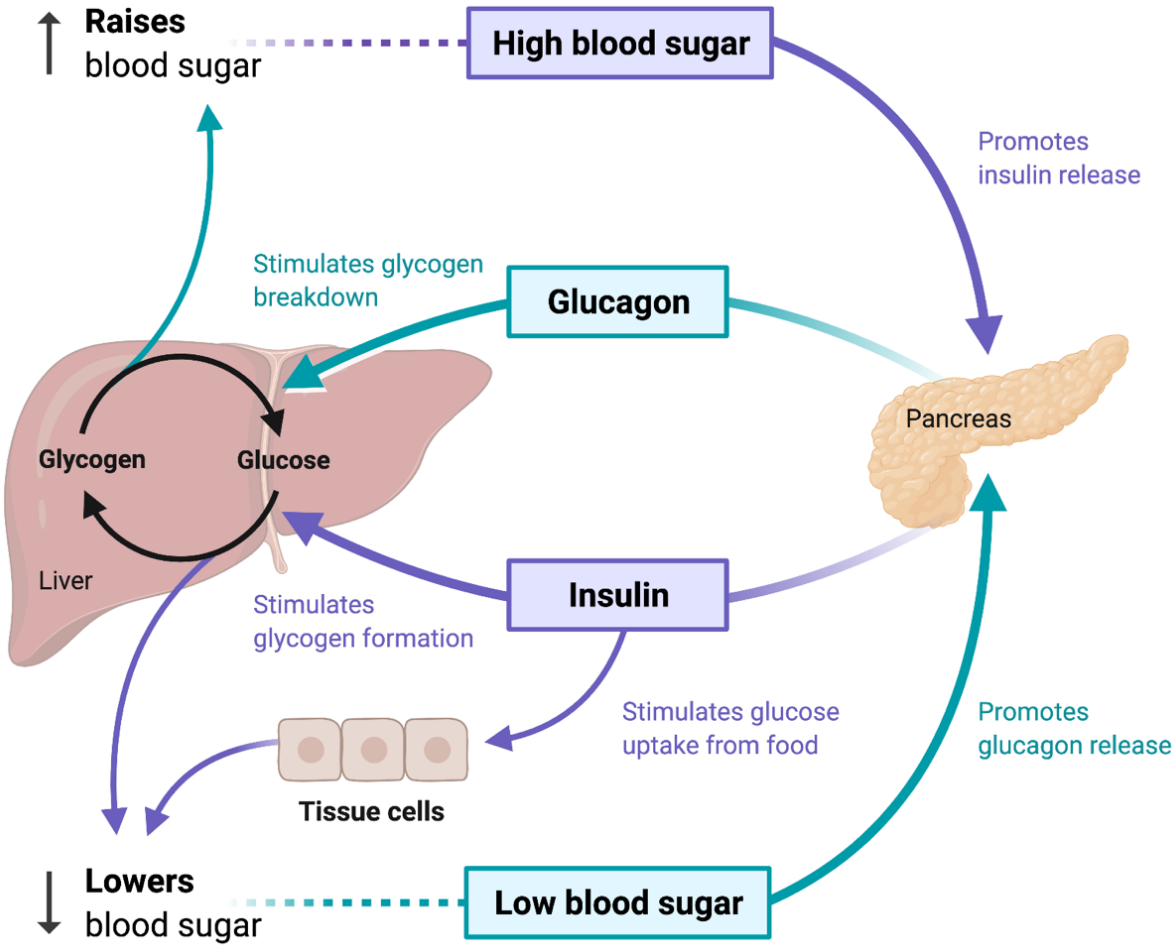


Figure *illustrating the regulation of blood glucose levels, simplified to include only glucagon as the main counter-regulatory hormone to insulin. One way to think of* ***glucagon*** *and* ***insulin*** *is also as a distinction between the* ***fasted*** *and* ***fed*** *state. In the fasted state, the liver is the primary site of glucose production, and glucagon stimulates the breakdown of* ***glycogen*** *[the stored form of glucose in cells] to release glucose, maintain blood glucose levels and supply glucose to the brain and skeletal muscle. However, during the postprandial period the production and release of glucose from the liver is suppressed by up to 80% over the 4 to 5hr after a meal – this is because there is glucose coming into the bloodstream from the diet, and the body does not need to produce more glucose while it disposes of dietary glucose intake. High blood sugar promotes insulin release from the pancreas, which then disposes of glucose into tissues, thus lowering blood glucose levels.*

Where does glucose go? If we take a hypothetical example of glucose disposal after a meal containing 100g of carbohydrate as glucose. As the 100g enters circulation from the small intestine, the first port of call is the liver where around 30% of that glucose is taken up in liver cells as glycogen. The remaining 70g goes on to enter into general circulation, where around 25-30% is taken up next by skeletal muscle, 5-15% by adipose (fat) tissue, 8-10% by the kidneys, and 15-20% taken up directly by the brain. A further 7-15% continues in circulation, and can be taken up again by the liver and other tissues, or stored in adipose tissue. Low blood glucose is technically more dangerous than high: if blood glucose level fall below 36mg/dL for just 5mins it may cause lethal brain damage, while blood glucose levels can be elevated for months before complications of diabetes may start to occur.

*Geek Box: Measures of Blood Glucose and Terminology

*So, let's spell some terms out here so you're on clear terms of what we're talking about. Let's start with **glycaemia**, which simply means the blood glucose level, thus **postprandial glycaemia** means the levels of glucose in the blood after a meal. Then we have **glycated haemoglobin A1c [HbA1c]**, a marker for longer-term blood glucose regulation over the period of the previous 3-months and is expressed as a percentage. Currently, 6.5% is considered the threshold for a diagnosis of T2D, and the range of 6.0–6.4% is considered 'high-risk' according to World Health Organisation guidelines. There is also a concept important for the present study, known by the broad definition of **glycaemic variability [GV]**. In simple terms, GV is the swings in blood glucose levels that occur throughout the day. GV includes the elevations in blood glucose that occur after a meal, the time spent with high or low blood glucose levels, and the difference in blood glucose responses at the same time of day, on different days. There are several measures of GV, but the present study used the **mean amplitude of glycaemic excursion [MAGE]**, which is a measure of the glucose excursions in response to meals that exceed 1 standard deviation from the average response. It also calculated the **mean of daily difference [MODD]**, which expresses how much the average variation in glucose on one day compares to the next [i.e., a low MODD indicates consistent glucose variability from day to day, a good thing!]. Finally, the **homeostatic model of insulin resistance [HOMA-IR]** is calculated from fasting insulin and glucose measures, based on higher fasting insulin levels responding, in a feedback loop, to higher fasting glucose levels, indicating peripheral insulin resistance to glucose uptake.*

The Study

The study was an open-label [i.e., both researchers and participants knew they were receiving the intervention], randomised controlled trial using a crossover design. Participants were females with insulin resistance [defined by HOMA-IR], who resided in the hospital where the intervention was delivered for the duration of the study, which was 21 days.

Diet was fully controlled by the researchers and the study compared two diets:

- **High Protein [HP]**: 30% protein, 40% carbohydrate, 30% fat.
- **Mediterranean Diet [MD]**: 20% protein, 55% carbohydrate, 25% fat.

Both diets were designed to achieve a 500kcal per day energy deficit.

As a crossover design, all participants received both diets. Therefore, randomisation is used to determine the order of diet sequence, i.e., who starts with MP followed by HP and vice versa. Each diet was consumed for 10 days.

The primary outcome was insulin levels and insulin resistance [measured by HOMA-IR]. The study also assessed measures of glycaemic variability [more under ***Key Characteristic***, below]. An exploratory analysis was conducted to determine whether baseline gut bacteria composition was associated with differences in glucose variability. The results are presented as mean with 95% confidence intervals.

Results: 16 women completed the intervention. 11 women were randomised to the diet sequence HP>MD and 6 to MD>HP; all 11 women randomised to the HP>M sequence completed the study, while 5 completed the study from the M>HP sequence.

- ***Fasting Insulin and Glucose Levels*:** On the HP diet, fasting insulin levels decreased by 3.5uU/mL (95% CI, –8.2 to 1.2uU/mL), while on the MD there was an increase of 1.5uU/mL (95% CI, –1.0 to 4.1uU/mL). When factoring in the diet sequence and treatment period, the average difference on the HP diet was 6.9uU/mL (95% CI 1.6 to 12.3uU/mL) lower fasting insulin compared to the MD. There was no significant difference in fasting glucose levels, an average difference of 0.17mg/dL in favour of the HP diet.
- ***Insulin Resistance [HOMA-IR]*:** On the HP diet, HOMA-IR decreased by 0.9 (95% CI, –0.1 to 2.1) compared to an increase of 0.3 (95% CI, –0.3 to 0.9). When factoring in the diet sequence and treatment period, the average difference on the HP diet was 1.7 (95% CI 0.5 to 3.0) lower HOMA-IR compared to the MD.

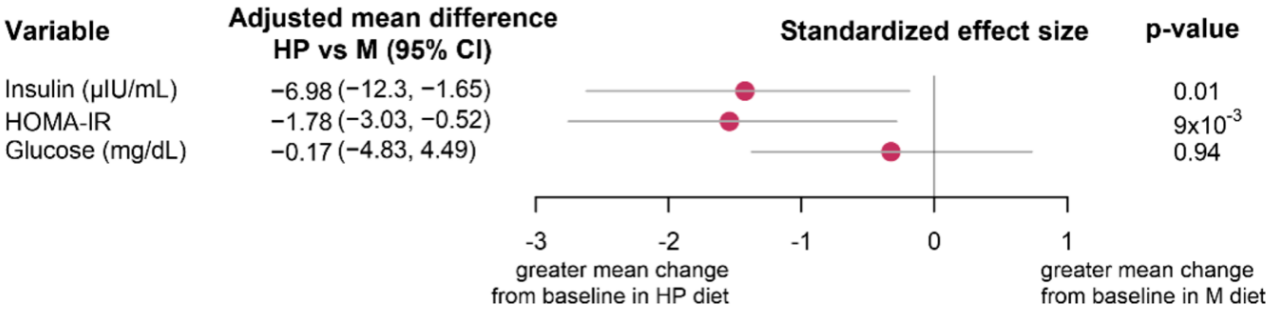


Figure from the paper showing the adjusted (for diet sequence and treatment period) difference between diets, with the HP diet showing a large effect on fasting insulin and HOMA-IR compared to the MD.

- ***Glycaemic Variability*:** There was no difference in daily glucose levels measured by CGM. However, the standard deviation of glucose was 3.13mg/dL (95% CI, 1.6 to 4.6) lower in the HP group, while the MAGE was 5.0mg/dL (95% CI, 1.1 to 9.0) lower, and MODD 1.4mg/dL (95% CI, 0.05 to 2.9) lower.

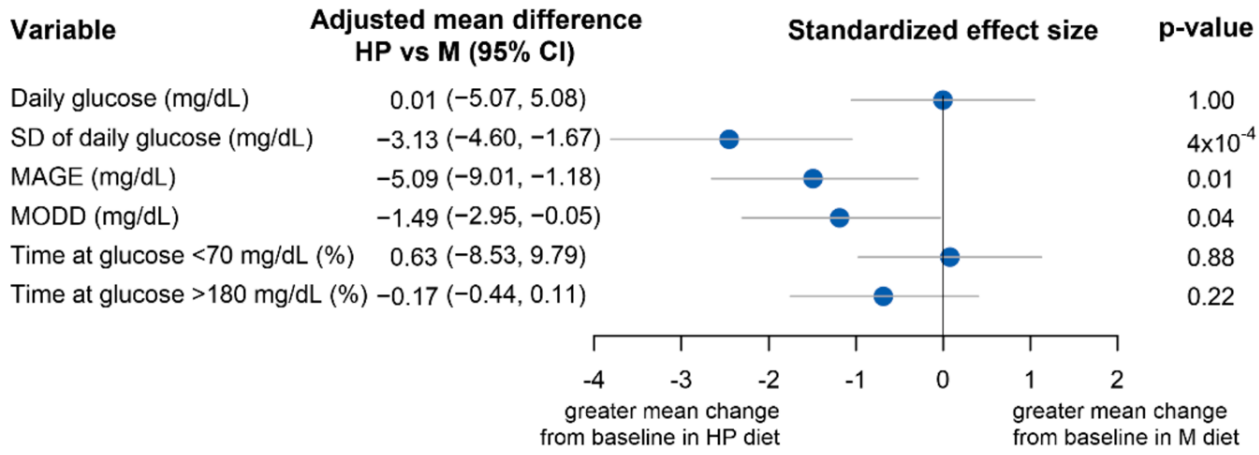


Figure from the paper showing the adjusted (for diet sequence and treatment period) difference between diets on various measures of glycaemic variability [more under Key Characteristic, below].

The Critical Breakdown

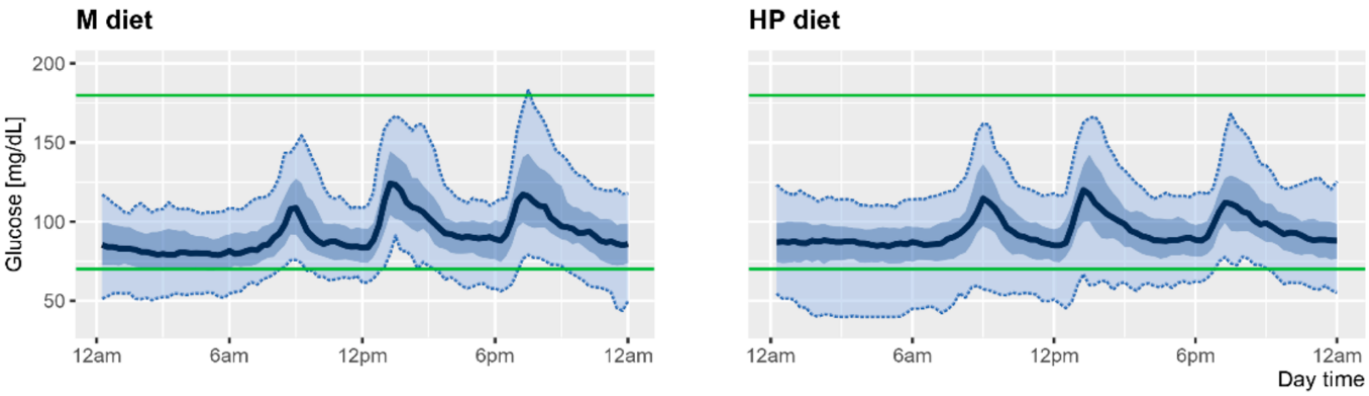
Pros: As with any dietary intervention, control of the diet is a critical factor and the major 'Pro' for this study is that the diets were fully controlled and served to participants in an in-patient setting. Although the diets carry different labels, the HP diet was in fact a very Mediterranean diet, only higher in protein; the study foods were similar between both diets, with the serving sizes manipulated to alter the macronutrient composition to each respective diet. Energy intake on the diets was tailored to individual requirements, based on measurements of resting energy expenditure.

Cons: The randomisation method was shoddy, and the paper states participants were randomised in 1:1 manner, but still ended up with largely imbalanced groups for diet order [n = 11 vs. n = 6]. Not only that, but the participants were not balanced according to relevant potential covariates: those in the HP>MD diet sequence group were 6yrs younger, 12kg lighter had a BMI 6-points lower, lower fat-to-lean mass ratio, and lower insulin secretion and insulin resistance. The authors state that these differences were "not statistically significant". Ah yes, the absolution from thinking bestowed by the p-value! C' mon. A more robust randomisation method could have stratified participants to ensure diet orders were balanced for covariates and numbers. This is relevant because, although they adjusted for diet sequence in the analysis, they didn' t adjust for these covariates, so the HP diet may have had a headstart on benefits given the baseline characteristics of this group. There was no washout period, yet the differences in the outcomes were calculated against the start of *each* diet, i.e., the start of the second diet was the end of the first. The diets lasted 10 days each, and the sample size is very small, thus beware of apparently large effect sizes.

Key Characteristic

The study used continuous glucose monitors [CGM] to assess glycaemic variability [GV]. CGM' s are little devices inserted into subcutaneous fat tissue [often lower abdominal fat], which measure interstitial glucose levels every 5min across a full 24 h day, and can be worn day-to-day. Note: CGM' s do not measure plasma glucose, but glucose entering interstitial fluid, a fluid which surrounds cells in the body. This means they are providing a measure of glucose from a different bodily compartment to plasma. So, what interstitial fluid glucose represents is glucose that is in the process of being transported to cells, while plasma/blood glucose reflects available circulating glucose.

One of the major advantages of CGMs is that you can illustrate glucose patterns across an entire day. The figure below is an example of this, taken from the present study, and illustrates the fluctuations in response to meals [the three 'peaks' that you can see], and then through the night. This means CGMs may capture a more dynamic picture of glucose levels, while plasma glucose is often measured only over a 2hr postprandial period.



Here is the thing we still don' t *really* know about the utility of measures of GV. Some authors have suggested that high GV influences factors like oxidative stress and endothelial dysfunction, and may capture risk for cardiovascular disease that measures like fasting glucose and/or HbA1c may be insensitive to capturing [8]. And in participants with type-2 diabetes [T2D], but controlled according to HbA1c levels, GV [specifically, the MAGE] was associated with diabetic neuropathy [9]. However, in participants with type-1 diabetes, there was no association between any measure of GV and vascular complications once average glucose levels were accounted for [10].

In the present study there was no effect of diet on either fasting plasma glucose or total daily glucose measured by CGM. However, there were differences for GV measures, and thus the relevant question is to what extent such improvements in overall GV would lead to meaningful reduction in risk of clinical endpoints, i.e., diabetes or cardiovascular disease outcomes. Indeed, a question is also whether there is any relevance to measures of GV independent of average glucose levels.

Interesting Finding

There was no effect on average glucose levels, but a reduction in insulin and in HOMA-IR. Why? It could be because it was fasting measures of glucose were assessed. A meta-analysis of interventions investigating the effects of high protein diets on glycaemic control found that high protein diets led to significant reductions in HbA1c, but had no effect on fasting glucose levels [11]. This may because the benefit of higher protein intakes on glycaemic control may relate primarily to its influence on postprandial glycaemia [4,5].

In the present study, if the average glucose levels were the same, but the GV was improved, it may suggest that the postprandial glycaemia was improved by the HP diet, which would be consistent with previous research [4,5]. The fact that fasting insulin and HOMA-IR decreased, but without an effect on fasting glucose, would also suggest that the improvements in glycaemic control reflect the postprandial period. But this is speculation, because the study did not ultimately analyse postprandial glucose.

Relevance

We could be tempted by the labels given to the respective diets to say, *"a Med diet has no effect!"* But we shall keep that powder dry, because in fact the foods making up both diets were basically the same. In effect, the major difference in the diet is not the foods, but the macronutrient composition, in particular the higher protein and lower carbohydrate combination [as dietary fat was similar between diets].

If we conceptualise the difference in diet as one primarily of protein and carbohydrate, there is some wider context we can place this study into. In the OmniHeart trial, a controlled weight-maintenance feeding study, subjects with prehypertension or hypertension were randomised to one of three modified DASH diets: one carbohydrate based [58% carb /15% protein /27% fat], a protein-based diet [48% carb /25% protein /27% fat] and a monounsaturated fat-based diet [48% carb /15% protein /21%MUFA (37% total fat)] [12]. Both the protein and MUFA diets led to significant reductions in blood pressure, blood lipids, and triglycerides compared to the carbohydrate diet [which still had benefit, to a lesser degree] [12].

In fact, cumulatively the data suggest that both protein and MUFA exert beneficial effects on glycaemic control [11,13]. This may be relevant for the population sampled for the present study, i.e., insulin resistant but not yet at a diagnosis of T2D, as it appears that postprandial glucose levels are a stronger determinant of longer-term blood glucose regulation, as measured using HbA1c, than fasting measures [14].

Bear in mind that the normal insulin reference range is ~7-10uU/mL, thus the reduction of 3.5uU/mL on the HP diet in the present study could be clinically meaningful. However, the wide confidence intervals (95% CI 1.6 to 12.3uU/mL) for the adjusted average difference suggests a lot of individual variability in this small sample size. This is where sample size is relevant, because the suggested effect size here is very large, an average lower fasting insulin on the HP diet of 6.9uU/mL. Hedging bets on regression to the mean, I would not be surprised if a larger study with more statistical power found a more modest effect size. But hey, that' s just me being sceptical.

Finally, you may recall they conducted an exploratory analysis on the associations with baseline microbiota composition and GV. They identified 5 bacterial families associated with positive GV control, and 5 associated with negative GV control. But the relevance of these is purely speculative, and I wanted to keep this Deepdive more glucose-focused.

Application to Practice

If we examine the diet composition, we can see that this intervention really provides a corroboration of the wider evidence on dietary manipulations and glucose regulation. In this respect, the limitations of the present study alone would not be sufficient to draw any conclusions beyond the caveat of *"interesting, more research needed."*

Except, there are larger controlled interventions, like OmniHeart, other DASH modifications, and interventions specifically investigating the effect of high-protein and/or MUFA-rich diets on glycaemic control [4,5,11–13]. From these data, we can conclude that targeting higher dietary protein intake in both men and women with impaired glucose tolerance, and sprinkling some olive oil on top, is a low-hanging fruit to pick in managing metabolic health and risk.

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