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Davis R, Bonham MP, Nguo K, Huggins CE. Glycaemic response at night is improved after eating a high protein meal compared with a standard meal: A cross-over study. Clin Nutr. 2020 May;39(5):1510-1516.

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

Disruption to circadian rhythms is increasingly recognised as a risk factor for metabolic disease [1]. While there is research demonstrating that night-shift work poses a significant risk for chronic lifestyle and metabolic disease [2-4], little is known about the optimal - if any - nutritional strategy for night-shift workers.

The most well-established circadian variation in metabolism is in relation to glucose tolerance [5]. Glucose tolerance exhibits strong variability over the day, with glucose disposal amplified in the early daytime and reduced in the evening [6,7]. In the evening, both increased post-prandial glucose and lower insulin responses are observed, resulting in prolonged elevation of blood glucose into the night [6,7]. However, while impaired insulin function is observed in the evening, it appears that the profound decreases in glucose tolerance observed are primarily mediated by reduced insulin sensitivity in peripheral tissues, in particular skeletal muscle tissue [6,7].

This decrease in glucose tolerance in the evening may, in part, explain the strong associations between evening/nighttime energy intake and adverse metabolic health. In particular, night-shift workers have an increased risk for type-2 diabetes [3,4]. The pattern of energy intake is also a relevant consideration. Recent research has shown that nurses working consecutive night-shifts redistribute energy intake to the nightshift phase between 18.00-07.00hrs [2,8]. Greater redistribution of energy intake to the nightshift was predictive of worse blood cholesterol levels [11].

With regard to macronutrients, protein intake has been shown to be lowest macronutrient, while carbohydrate the highest, during the night-shift period [8]. While much of the focus in this area has related to rhythms in glucose and fat metabolism, little is known about the metabolic effects of protein ingestion during the biological night. However, a proof-in-concept study using nasogastric feeding of 40g casein protein overnight in elderly men found normal protein digestion and absorption kinetics, indicating that the gut may function at night in response to protein ingestion [9]. Further, higher dietary protein intake is known to have beneficial effects on glucose excursions and glucose tolerance [10,11].

Could high protein meals be beneficial for metabolic health in the context of evening energy intake?

The present study investigated this question.

The Study

10 healthy completed a 2 x 2 factorial cross-over design* intervention, comparing the effects of a high-protein meal vs. a standard meal consumed at 8am vs. consumed at 8pm, with each subject crossing over to both meals, at both time points [i.e., each participant consumed each of the four test meals]. Test meals were each separated by an average of 8-days.

Participants were advised to maintain a consistent sleep schedule for three days prior to each meal test. Before each test meal, participants consumed a calorie-matched control meal ~10hrs in advance consisting of 44% carbohydrate, 36% fat, and 17% from protein, and then fasted until the test meal, i.e., overnight in the 8am test meal condition or from 10am in the 8pm test meal condition.

The test meals consisted of the following:

- ***Standard Meal [SM]:*** 15% protein, 35% fat [14g saturated fat], 46% carbohydrate [33g sugars, 12g fibre].
- ***High-Protein Meal [HPM]:*** 41% protein, 29% fat [4g saturated fat], 29% carbohydrate [23g sugars, 21g fibre].

In the fasted state prior to each test meal, participants underwent body composition measures and had fasting blood samples taken. The test meal was provided at the intended time, following which blood samples were collected every 15mins for 180mins. The primary outcome was postprandial blood glucose levels, and the stated secondary outcome was hunger and fullness. Other outcomes including insulin, blood pressure, and body composition measures.

*Geek Box: 2 x 2 Factorial Design

A 2 x 2 factorial design is a specific trial design which tests two interventions in the one study sample. In a 2 x 2 design, there are two [or more] independent variables, and a dependent variable. The 'factor' is the independent variable. Each factor may have different levels. Therefore, in a "2 x 2" design, there are two independent variables [factors] and two levels of each factor, e.g., two separate time points. For example, you could want to test two different drugs which target a similar outcome, e.g., test a statin [independent factor No.1] vs. placebo and a PCSK9-inhibitor [independent factor No.2] vs. placebo for their effects on lowering LDL-cholesterol [dependent variable], and also test the effect of Dose A [level No.1] or Dose B [level No.2] for each drug. Thus, we have 2 factors [each drug] and 2 levels [different doses of each drug]. So you would have participants randomised to Statin-Dose A, Statin-Dose B, PCSK9-inhibitor-Dose A, PCSK9-inhibitor-Dose B: 2 x 2. There are a number of results you can get from this type of design. You could main effects and/or interaction effects. The 'main effect' is an outcome related to the levels of the factor. In our hypothetical example, there could be a main effect of the dose if the drug had an effect at each level of the dose. There could also be a main effect of drug if we found a difference between drugs that was independent of dose. You could also have an 'interaction effect', e.g., it could be that the combination of the PCSK9-inhibitor drug plus Dose B improves the outcome better than the other combinations. To bring this back around to the present study, we can see that each participant consumed both the standard test meal [factor No.1] and high protein test meal [factor No.2] at 8am [level No.1] in the morning and 8pm [level No.2] in the evening, for a total of four test meals. The present study also utilised a cross-over design, meaning that each subject served as their own control and consumed each of the four test meals. Cross-over designs are useful for nutrition interventions, given that there may be distinct inter-individual differences in metabolism and responses to a particular exposure [either diet or supplement]. Factorial designs - whether 2 x 2, 2 x 4, etc. - are helpful trial designs which allow for different independent variables [the factors] to be included in a single study, so they are an efficient way of doing research. They also allow for interaction effects to be examined, which is important in determining whether differences in treatment may be explained by variations between the factors and levels examined.

Results: Participants included 5 female and 5 male subjects, aged 19-33yrs [median 22yrs] with an average BMI of 22.6 and fat mass of 29.9% and 12.9% in the female and male participants, respectively. The fasting glucose and insulin values before each test meal, at each time of day, were similar between groups.

- ***Effect of Meal Time on Glucose Response:*** Both the SM and HPM resulted in significantly elevated glucose levels at 8pm compared to 8am. Thus, independent of test meal there were greater postprandial glucose levels in the evening compared to the morning. However, the magnitude of the difference was significantly greater in the SM group, with postprandial glucose levels 36.4mmol/L after 8am vs. 208.8mmol/L after 8pm. In the HP group, postprandial glucose levels were 12.9mmol/L after 8am vs. 59.6mmol/L after 8pm.

- ***Effect of Test Meal on Glucose Response:*** In the evening 8pm test meal, compared to the SM the HPM resulted in a significant 74.1% lower postprandial glucose response [59.6mmol/L vs. 208.8mmol/L]. The difference between meal types in the morning 8am test were not statistically significant, however, the HP meal resulted in 64.6% lower postprandial glucose compared to the SM.

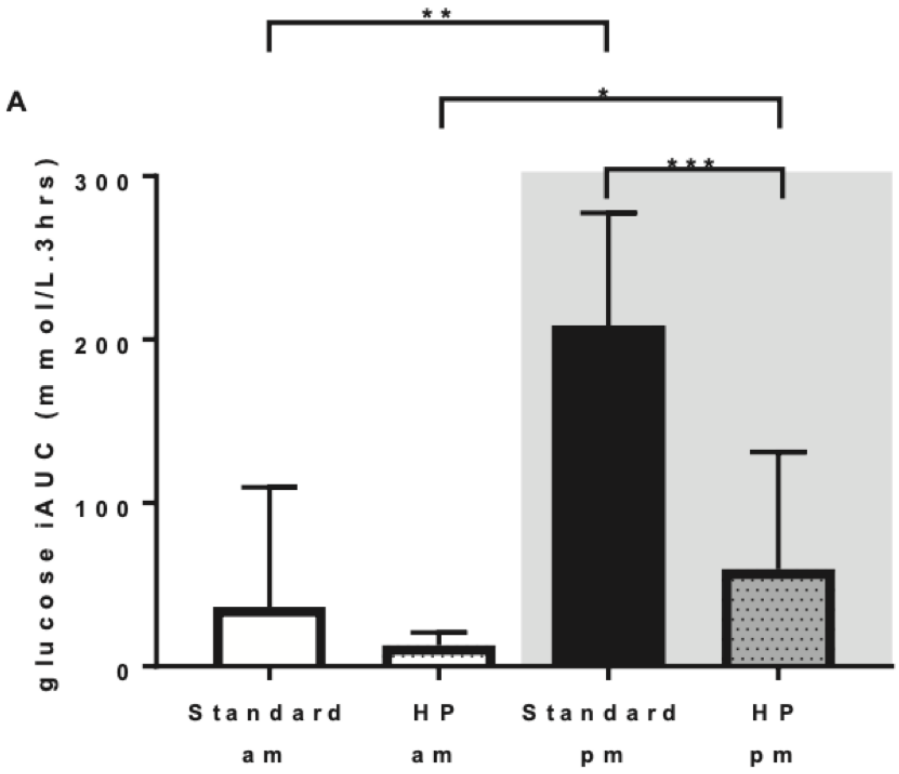

Figure *from paper illustrating the difference between the standard meal [SM] and high-protein meal [HPM] consumed at both 8am [left bars] and 8pm [right bars with shaded grey background] in the glucose incremental area under the curve. The 'iAUC' is the sum of the elevations in glucose over the entire time period measured [in this study this was 3hrs], over and above the baseline value. While the blood glucose response to the HPM in the evening was statistically significantly greater than the HPM at 8am, the overall iAUC was still <60mmol/L/3hrs. Conversely, the glucose response to the SM test at 8pm was 82.6% higher compared to the 8am SM test meal. Between the two meals, the glucose response to the HP meal at 8pm was 74.1% lower than the SM meal.*

- ***Effect of Test Meals and Time on Subjective Appetite:*** There were no significant differences in fullness scores between the HP and SM meals, at either 8am or 8pm. There was a trend toward less hunger in the evening following the HP meal vs. the SM meal, but this difference was not statistically significant.
- ***Effect of Meal Time and Type on Insulin Response:*** There were no significant differences in insulin responses between the SM and HPM test meals, at either time of day. Insulin levels were higher in the evening following the SM meal compared to the HPM test meal, but this was not statistically significant. HOMA-IR, a measure of insulin resistance, was significantly higher in the morning compared to the evening, although there was no significant difference between the two meal conditions.

The Critical Breakdown

Pros: Both the control meals and test meals were matched for total energy intake, and the control meal was identical in macronutrients. The test meals were prepared by the investigators and weighed on electronic scales. Participants were blinded to the test meals. The fasting duration between the controlled pre-meal and the test meal were similar, mitigating any potential carryover effects from either the meal or the fasting period. While the sample size is small, the published power calculation indicates that a minimum of seven participants were required for the primary outcome of blood glucose, thus it appears the study had adequate statistical power to detect this effect.

Cons: The study meals were not matched for key nutrients known to impact glucose responses, in particular fibre, sugars, and saturated fats. Thus, it is not possible to determine the effects of protein content of the meals alone. However, the comparison to the 'standard' meal which reflected a typical 'Western' diet pattern in macronutrient composition was still a valid comparison, it just warrants caution in assuming the entire effect is mediated by protein. The sample size was small and participants were otherwise in clean bills of metabolic health, therefore the findings may not generalise to at-risk populations, in particular night-shift workers. From the perspective of shift work, 8pm is not 'late', and it would have been incredibly valuable to have an additional level for time at, for example, 1am [but no study is perfect, so...].

Key Characteristic

The use of a truly very high-protein diet intervention has potentially important implications for shift work, although as stated in the 'Cons' above, we can't say the entire effect was due to protein with important nutrient covariates like fibre, sugar, and saturated fat not matched between diets. But this study provides a step toward in a very under-researched area. The literature in relation to protein timing during the night largely comes from sports nutrition, where numerous interventions have investigated the effects of pre-sleep protein ingestion to support muscle protein synthesis and recovery in healthy young men [(12)]. The metabolic fate of dietary protein during the biological night has been demonstrated via stable isotope-labelled protein, which showed that proteins were incorporated into skeletal muscle during the night [(13)]. There is evidence from other stable isotope-labelled protein feeding research that protein metabolism during the night remains relatively normal [(14)]. These studies provide support for a profile of dietary protein digestion and utilisation during the biological night distinct from that of carbohydrate or fat. High-protein, low-carb and low-fat feeding during the night may provide a nutritional strategy to attenuate the adverse effects of eating during night shifts, but to date such an intervention has not been undertaken.

Interesting Finding

Rather bizarrely given the health of the participants, the HOMA-IR scores in both the morning and evening indicated significant insulin resistance, which was higher in the morning - the opposite of what we might expect. Homeostatic Model Assessment of Insulin Resistance [HOMA-IR] is a commonly used method to assess insulin resistance using a calculation based off fasting blood glucose and insulin values. Simply put, HOMA-IR provides a a measure of how hard insulin is working to keep blood glucose levels in a normal range. The calculation for HOMA-IR is based on mathematical modelling, and a HOMA-IR score is derived by multiplying fasting blood glucose levels by fasting insulin levels, and dividing by a factor of 405 if using mg/dL or 22.5 if using mmol/L. The mathematical model is based on endogenous fasting glucose levels being regulated by the capacity of pancreatic beta-cells to produce insulin in response to blood glucose concentrations. A score of 1.0 and range of 0.5-1.4 indicates normal insulin sensitivity; above 1.9 indicates early insulin resistance, while over 2.9 indicates significant insulin resistance.

In the present study, HOMA-IR in the morning was 3.4 and 3.2 in the SM and HPM, respectively, indicating significant insulin resistance! In the evening, HOMA-IR was 2.0 and 1.7 in the SM and HPM, respectively, indicating early insulin resistance. So, could these lean participants with normal waist circumference and very low visceral fat levels be on a ticket to type-2? Maybe not. HOMA-IR is a very useful and well-validated model, but it does have limitations, one of which is in lean participants. A study comparing HOMA-IR to the gold standard laboratory method of assessing insulin resistance, the euglycemic-hyperinsulinemic clamp, found that HOMA-IR correlated poorly with the clamp over 3-hrs in participants with a BMI <25 and normal glucose tolerance [15]. Given all of the other markers in the study sample that we would expect to correlate with insulin resistance - fasting glucose, lean mass, fat mass, visceral fat, waist circumference - were all practically perfect, it could be that the HOMA-IR score is a 'red herring' . Oddly, the authors did not give this finding any discussion.

Relevance

Effective nutritional strategies for shift work remain lacking. Although this study was not technically testing a night-shift protocol, it provides initial proof-in-concept of a beneficial effect of high-protein meals in the evening on postprandial glucose levels, which has potentially important implications for night-shift work. An interesting observation in the literature is that night shift workers consume the same amount of total daily energy as fixed day workers [16]. Therefore, strategies which manipulate macronutrient content may be more effect than attempting to alter total energy intake. There is a small but consistent body of research indicating that overnight low-calorie, protein-rich feedings low in carbohydrate and fat may, at the very least, not be detrimental for glucose and fat metabolism [9, 12-14]. However, the crucial limitation of the literature on overnight protein intake to date is that protein is ingested pre-sleep, and consequently these studies do not provide any insight into whether extended wakefulness, required for night-shift work, would alter the kinetics of protein digestion and absorption, and the interaction with blood lipid and glucose regulation.

Conversely, while the comparison 'Standard Meal' was not matched for macronutrients, the fact that it resembled a typical 'Western' macronutrient profile is itself insightful from a shift work perspective. This is because the metabolic effects of one meal are not independent of energy intake at previous or subsequent meals [17]. A number of studies have shown that the macronutrient content of an evening meal may impact on the metabolic response to the next morning meal, depending on the dominant macronutrient in a previous evening meal [18-20]. For example, Robertson et al. demonstrated that the postprandial triglyceride response following an oral fat tolerance test in the morning was significantly elevated when the previous evening meal was high in carbohydrate [18]. In another study testing glucose responses in healthy young males to breakfast eaten between 06.30-07.00hrs, consuming 40% daily energy at 01.30hrs resulted in a significantly increased glucose response and impaired insulin sensitivity [21].

It may be that the deleterious impacts on circulating lipids and glucose tolerance during the biological night may be mediated or attenuated by protein-based feeding. This may in turn be mediated by the macronutrient composition of the main meal preceding nightshift work. However, there is a lack of data on primary cardio-metabolic risk factors, including blood lipids and blood glucose regulation, and the effects of dietary protein feeding during what would reflect a night-shift, i.e., ~9pm-7am, has not been investigated.

Application to Practice

The limited research on protein metabolism during the biological night is suggestive of a potentially novel nutritional strategy for shift workers, which could include food-based recommendations for protein-based snacks at particular time-points. It could be helpful advice to consume a high-protein, lower carb/fat meal as the main meal prior to a night-shift. Whether the same composition would also result in more favourable effects if consumed at, say, 1am or 4am, remains to be tested. However, in the spirit of 'absence of evidence' and the practical reality that night-shift work is not going anywhere, then at the very least the evidence does support minimising carb and fat-rich foods during the biological night.

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