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Smith K, Taylor GS, Allerton DM, et al. The Postprandial Glycaemic and Hormonal Responses Following the Ingestion of a Novel, Ready-to-Drink Shot Containing a Low Dose of Whey Protein in Centrally Obese and Lean Adult Males: A Randomised Controlled Trial. *Frontiers in Endocrinology (Lausanne)*. 2021;12:696977.

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

We tend to have definitive characterisations of disease states and risk, and this can lead us into thinking about risk in more binary "have/have-not" terms. For metabolic disease, however, we know this is not the case: glucose tolerance is a spectrum from normal glucose tolerance to impaired glucose tolerance and prediabetes, to a diagnosis of type-2 diabetes [T2D] [(1)].

We also know that, although the relationship is not linear, with increasing BMI there is an increased risk for the development of T2D [(2)]. However, BMI does vary substantially at time of diagnosis of T2D, which indicates that the risk is associated with underlying metabolic complications, including insulin resistance, and decreased pancreatic beta-cell function [(3,4)].

In fact, although we think of this spectrum as related to *glucose intolerance*, what makes an individual progress from impaired glucose tolerance to T2D is the progressive decline in the ability of the pancreas to produce and release insulin [(5)]. It is the constant struggling of the beta-cells to respond to elevated blood glucose that places this strain, and ultimately, failure, of the ability to produce insulin to lower glucose levels [(5)].

What factors influence post-prandial glucose levels? There are multiple factors which may influence the extent of postprandial glycaemia after a meal, including exercise and physical activity, time of day, time from the previous meal, and composition of the meal [(3)].

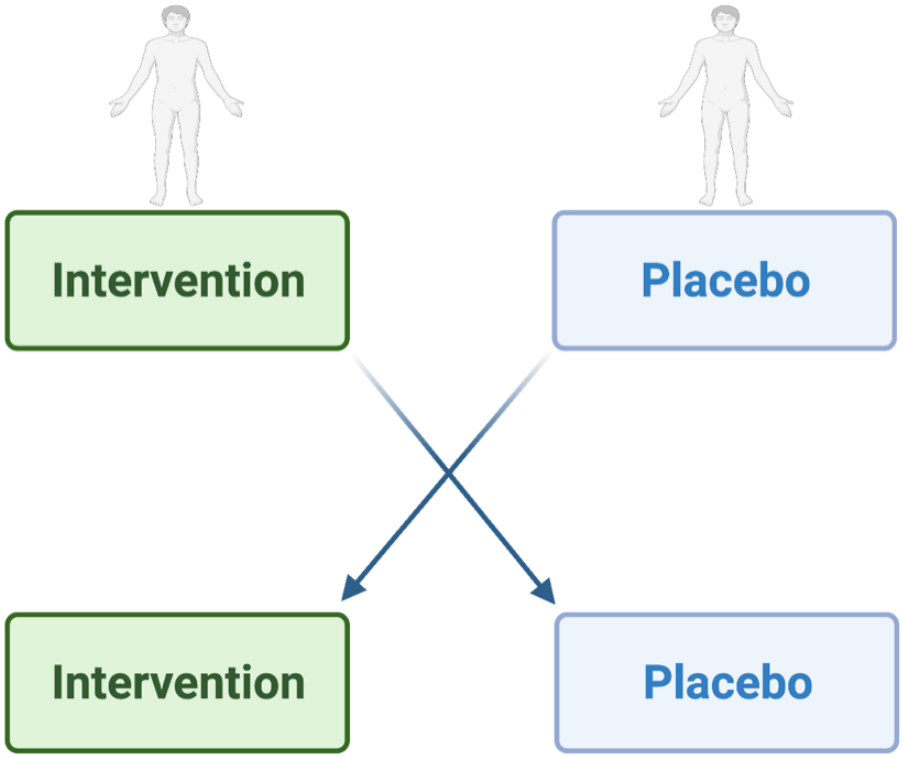
Within the latter – the composition of a meal – whey protein intake has attracted interest, due to the ability to stimulate incretin hormones* like glucagon-like peptide-1 [GLP-1] and glucose-dependent insulinotropic peptide [GIP], which in turn lower postprandial glucose levels [(6)]. The present study investigated the effects of a whey protein pre-load before breakfast on blood glucose responses.

*Geek Box: Incretin Hormones

The term "incretin hormones" describes GLP-1 and GIP, two hormones that are secreted by the gastrointestinal tract, specifically in the small intestine. These hormones are nutrient-sensing hormones and are secreted in response to the passing of food from the stomach to the small intestine. Both hormones stimulate the response of insulin to the elevation of glucose in the blood. Incretin hormones make the most significant contribution to the signalling for the secretion of insulin, even more than blood glucose itself. Although both GLP-1 and GIP exert this effect, it is important to note that they both have independent mechanisms. GIP primarily stimulates insulin secretion in a manner that is dose-dependent to the level of glucose in the blood [which GLP-1 also does]. However, GIP has no effect on gastric emptying, whereas GLP-1 does by slowing the rate of gastric emptying, , i.e., the rate at which food leaves the stomach into the small intestine for further digestion and nutrient absorption. GLP-1 also reduces gastric acid secretions and slows the overall rate of intestinal transit. This helps control the overall digestive process, but importantly, can thus also result in a slower rate of glucose absorption into the blood. It is thought that this role of GLP-1 provides a means of controlling overall gastrointestinal motility, to ensure the proper passage of food through the gastrointestinal tract. We also know that incretin hormones follow a circadian rhythm and are amplified in the morning, which may explain the beneficial effect on morning energy intake on postprandial glucose responses.

The Study

The study was designed as a randomised, placebo controlled, counterbalanced [where the order of treatments goes both ways] and crossover [where all participants are both treated and given the placebo] trial [see **Figure** illustration, below]. The trial recruited both lean participants and participants with obesity, to determine whether adiposity influenced the effects of the intervention.



The trial investigated the postprandial responses to breakfast following 15.6g hydrolysed whey protein delivered in a 100ml ready-to-drink shot, consumed 15min before breakfast. The placebo was 100ml of water. Participants consumed either whey or placebo 15min before being served a standardised breakfast of 60g Cheerio' s and 250ml whole milk [387kcal total; 58% carbohydrate, 27% fat, 15% protein]. Measures were taken before/after the drink, then for 240min [4 h] over the postprandial period. The **Figure** below illustrates the sequence of the test days.

The primary outcome was postprandial glucose in response to breakfast. Secondary outcomes included the acceptability and practicality of the whey shot, incretin hormones and insulin, and gastric emptying.

Results: 24 participants completed the study [12 with a BMI of 23; 12 with a BMI of 33]. All participants were male, with an average age of 35yrs. At baseline, fasting insulin was twice as high in participants with obesity compared to lean participants, and had twice as high insulin resistance.

- ***Postprandial Glucose*:** After the whey shot, postprandial glucose over 1 h after breakfast were significantly lower by 18.2% and 13% in the lean and obese participants, respectively, compared to the placebo. There was no significant different in total postprandial glucose levels over 4 h in either group of participants.

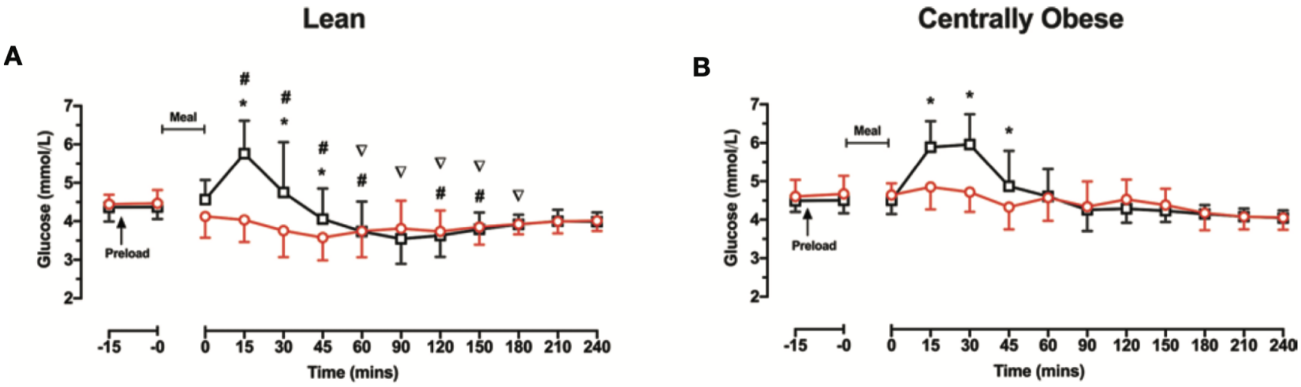


***Figure** from the paper illustrating the postprandial glucose responses in lean participants [**left**] and participants with central adiposity [**right**]. The **red line** indicates the whey shot pre-load, while the **black line** indicates the water placebo. As you can see, the effect of the whey pre-load was to suppress the postprandial glucose response, which on the placebo clearly spiked over the first 15-30min after breakfast, before declining by 60min such that there was no longer a difference in blood glucose levels from that timepoint on.*

- ***Postprandial Insulin*:** There was no difference in lean participants, however, participants with obesity had 3-fold higher insulin levels in response to the breakfast meal during both the whey pre-load and the placebo. Compared to the placebo, the whey pre-load in fact resulted in greater insulin secretion in the participants with obesity.

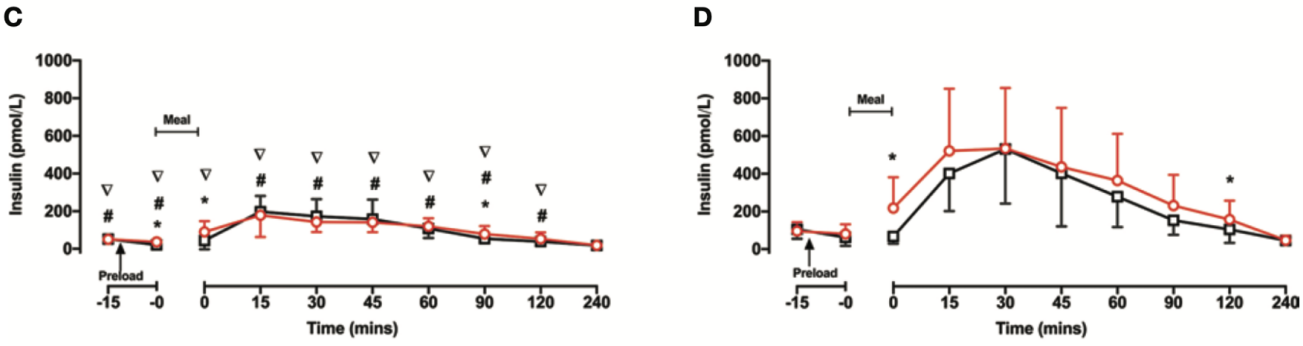
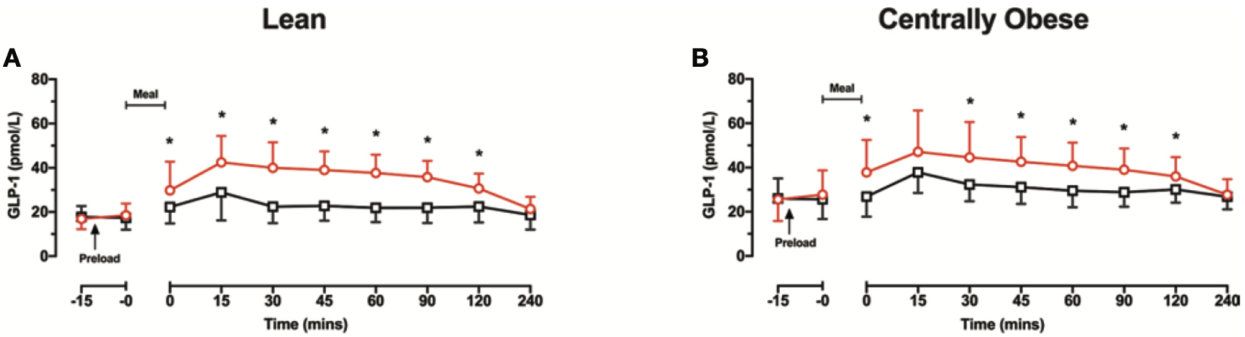


***Figure** from the paper illustrating the postprandial insulin responses in lean participants [**left**] and participants with central adiposity [**right**].*

- ***Postprandial GPL-1 and GIP*:** In both lean and participants with obesity, GLP-1 increased significantly by 2-3-fold over the full 4 h postprandial period [see **Figure**, below]. However, overall GLP-1 levels were 27% lower in participants with obesity. GIP after breakfast increased by 17.7% and 34.3% in the lean and obese participants, respectively, compared to the placebo.



- ***Whey Shot Palatability and Acceptability*:** The participants rated the taste of the whey ready-to-drink product at 76%, likability at 73%, and palatability at 77% [more under ***Key Characteristic***, below].

The Critical Breakdown

Pros: With the caveat that it would have been strengthened with single-blinding of participants, this study had a good design. The counterbalanced order meant that any potential effect of treatment order would be accounted for, i.e., an effect of all going first with whey before the placebo. And the crossover design meant that each participant served as their own control [i.e., the treatment effect is compared to the placebo effect in the same individual], which accounts for within-person error to influence the results. All participants were provided with a standardised dinner the night before each test day, which would minimise any carryover effects of different meal compositions and energy contents before testing. Both total and active GLP-1 were measured, which is a positive because only about 15-25% of total measured GLP-1 is biologically active [7]. The study really engaged with considering the applicability of the intervention in the real world [more under ***Key Characteristic***, below]. Having both lean and obese participants allowed for differences in response according to adiposity to be determined.

Cons: There is no description of the method of randomisation used for participants. The whey shot had very specific taste and texture characteristics, and thus having only water for the placebo would have been obvious. However, the trial makes no claim in its design to be blinded study, so this is less fatal than if the intention was to have participants blinded to their allocation. Nevertheless, the rigour of the trial would have been strengthened by having single-blinding, and matching qualities of the treatment and placebo drinks. The study enrolled no female participants. The sample size was right on the estimated power calculation, however, at 24 – and with 12 of each level of BMI – this is a small study. Gastric emptying was assessed by giving participants paracetamol, which is not broken down in the stomach, and measuring the rate of appearance of paracetamol in the blood. However, this is not a very refined method compared to other techniques, particularly the use of non-invasive breath tests [more under ***Interesting Finding***, below]. It is always worth noting that the study was industry-funded [by Arla, the dairy manufacturers who made the product], but there is nothing in the methods or outcomes that gives rise to any concerns in this regard.

Key Characteristic

It is refreshing to see a group of researchers really engage with generalisability. In general, intervention trials try to aim for *internal validity*, which is the term for methodological characteristics such as randomisation, double-blinding, placebo-control, etc., that are considered to reduce risk of bias and demonstrate cause-effect relationships.

However, achieving these types of design characteristics may mean that a study is able to achieve a more accurate finding, but does it mean that that finding would hold in real life? This latter question is *external validity*, also known as generalisability, i.e., the applicability of research findings in real life. In effect, internal validity asks, "*is this finding true?*", while external validity asks, *"who and where is this finding true for?"*

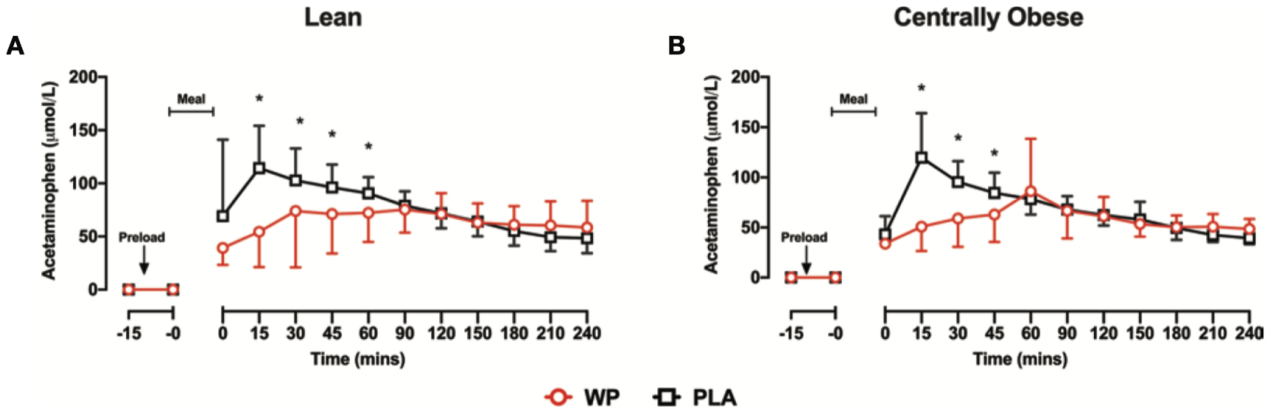
Many tightly controlled nutrition studies become difficult to generalise, because the artificial conditions of laboratories, novel interventions or very specifically controlled dietary intake, do not reflect free-living conditions. The present study noted in its introduction that the idea of people walking around with protein powder to mix up and drink in public is a barrier to the implementation of findings of the protein pre-load research. As a result, the research term worked in conjunction with the study sponsors, Arla, to produce the ready-to-drink shot of 100ml, containing the target dose of whey protein, to have the potential means to apply protein pre-loads in an everyday, real-life setting. The high ratings of palatability, taste, and likeability are thus very encouraging findings themselves.

Interesting Finding

Notwithstanding their use of a very unrefined measure of gastric emptying, there is some interesting data from this study on this outcome as it related to postprandial glucose responses.

Let' s start with the limitations of the test. As paracetamol is not broken down in the stomach, its rate of appearance in the blood is measured as a proxy for the rate of food leaving the stomach. However, although this can serve as a proxy for the rate of gastric emptying, the problem is that some paracetamol may leave the stomach faster than the rate of the meal' s transfer, but may then be slowly absorbed from the small intestine [8]. Thus, it is not as accurate a marker of gastric emptying as other methods.

With that caveat stated, however, let' s think about the findings. The first is that, in both lean and obese participants, the rate of appearance of paracetamol in the blood over the first 60min after breakfast was 35% lower [see **Figure**, below].



However, the finding of most interest here is that there was a correlation between the rate of appearance of paracetamol over 60min after breakfast with the corresponding postprandial glucose response over 60min. Why is this interesting?

Because recall from the ***Geek Box** above that gastric emptying rate itself influences postprandial glucose levels, i.e., the slower rate of food passing from the stomach to the small intestine limits the subsequent rate of glucose absorption to the blood [9,10]. We know from wider research that this feedback regulation of gastric emptying rate, and attenuation of postprandial glucose levels, is mediated by GLP-1 [11]. The correlation in the present study was weak [r = 0.31 on a scale of –1.0 to 1.0], which likely reflects the method used to assess gastric emptying, as other research using stable isotope tracers has found very strong [r = 0.89] correlations between gastric emptying and postprandial glycaemia [11].

Relevance

Overall, the findings in the present study are consistent with the wider research demonstrating that whey protein pre-loads are effective at reducing the magnitude of postprandial glucose responses [6]. This effect is observed in both participants with normal glucose tolerance and with T2D [12]. And this effect appears to be primarily mediated by the stimulatory effect of whey proteins on the incretin hormones, GLP-1 and GIP [10,12].

However, this novel intervention strategy is of particular relevance for states of impaired glucose tolerance to T2D. Why? Because loss of first-phase insulin response, i.e., the immediate response to elevations in blood glucose levels, is what characterises progressive beta-cell decline in T2D [5]. Restoration of first-phase insulin responses, indicative of restored beta-cell function, is the primary aim of interventions like the very-low-calorie liquid diets used by Professor Roy Taylor' s research group in the DiRECT study [13].

In between loss of beta-cell function and restoration lies preservation, and whey pre-loads augment the first-phase insulin response in adults with T2D, mediated by enhanced GLP-1 secretion [12]. The present study did not investigate T2D, but specifically compared lean and insulin sensitive participants to participants with central abdominal adiposity who were insulin resistant, which broadens the population subgroups in which this intervention may apply.

The beneficial effect of the whey pre-load in insulin resistant participants is particularly important. Bear in mind that in this study, there was little effect of the whey shot on insulin levels, despite modest elevations in GIP. This indicates that the lower postprandial glucose levels were not primarily achieved through GIP-stimulated insulin secretion.

However, GLP-1 was substantially elevated by 3-4-fold and gastric emptying was suppressed, which correlated with lower postprandial glucose. This indicates that the postprandial glucose-lowering was primarily independent of insulin. The inhibitory effect of GLP-1 on the rate of gastric emptying appears to be an independent influence on postprandial glucose levels [9,11,12]. Thus, this may become another consideration in the nutritional management of metabolic disease, beyond the usual hyperfocus on insulin.

Application to Practice

The concept of meal pre-loads using different macronutrients – mostly protein or fat ingested before carbohydrates – has been a novel experimental concept in research. However, the words 'novel' and 'experimental' have also carried the implication that these appeared to be the types of studies that would sit on shelves and never find their way into any sort of application.

After all, someone is hardly going to drink 30ml of olive oil before they eat their mashed potatoes, or carry 40g of glutamine handy to down before their work colleagues [6]. And whipping out a protein shake in public is a bit too Chad. A major advantage of the present study was the development of a very small [100ml] ready-to-drink whey "shot" in commercial packaging, that could have greater application in the real world.

For otherwise healthy individuals, is it worth eating the yogurt before the banana? Hardly. But for clinical contexts, if the type of product used were to become available for use in management of metabolic disease, it could be of real clinical utility.

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