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TABLE OF

CONTENTS

Hoover SE, Gower BA, Cedillo YE, Chandler-Laney PC, Deemer SE, Goss AM. Changes in Ghrelin and Glucagon following a Low Glycemic Load Diet in Women with PCOS. *J Clin Endocrinol Metab*. 2021;106(5):e2151-e2161.

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

In previous Deepdives, we have highlighted the complex and multifactorial presentations of Polycystic Ovary Syndrome [PCOS]. Much of our focus in these previous Deepdives was in relation to metabolic dysfunction [i.e., insulin] and hormonal dysfunction, i.e., androgens, leptin, and ghrelin.

In the most recent Deepdive on this topic, we focused in particular on leptin and ghrelin. Today, we're going to home in more on ghrelin. Leptin tends to occupy more focus for PCOS due to its more established role in regulating energy balance and potentially influencing hypothalamic-pituitary-adrenal-ovarian [HPAO] axis dysfunction [1,2].

However, it is possible that ghrelin also influences HPAO axis dysfunction, while also influencing energy balance through its role as a satiety hormone [3–5].

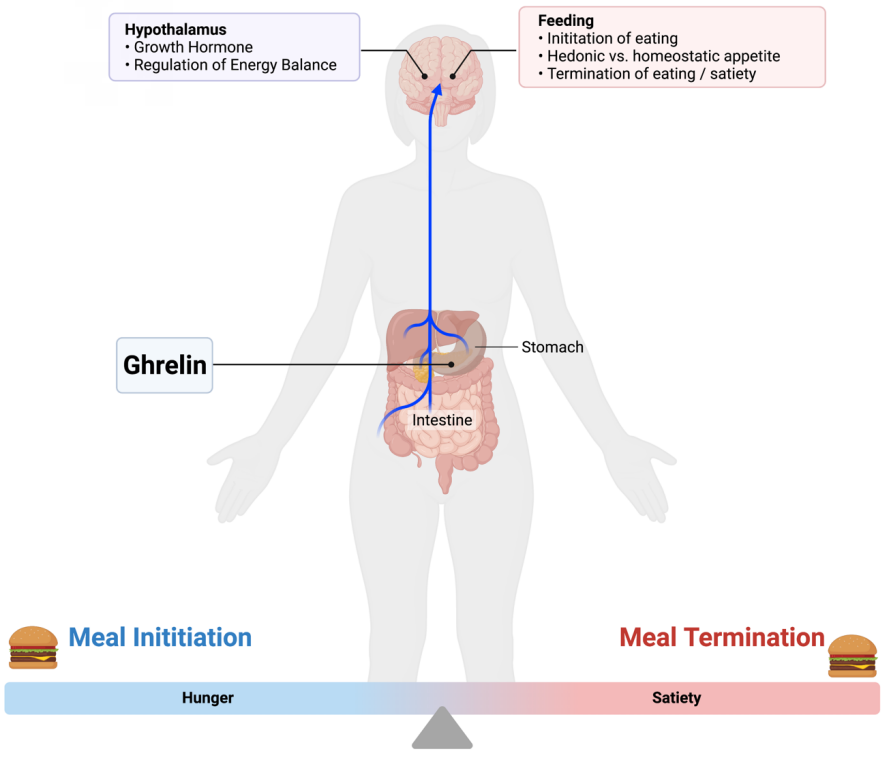

***Figure** illustrating certain of the neurological and hormonal roles for ghrelin. Ghrelin is secreted by stomach cells, and released when the stomach is empty. In this capacity, ghrelin is elevated with hunger and the rise in ghrelin precipitates the initiation of eating. However, ghrelin also influences energy balance through homeostatic [longer-term regulation of energy balance] and hedonic [short term hunger and appetite] regulation in the brain.*

Generally, ghrelin and leptin have an inverse relationship; hunger is associated with elevated ghrelin and lower leptin, while satiety associated with suppressed ghrelin and elevated leptin [1,3]. Women with PCOS typically show lower levels of ghrelin across a range of PCOS phenotype presentations [5]. The potential influence of dietary interventions on ghrelin levels is therefore of interest for the management of PCOS. The present study was a secondary analysis of a prior intervention.

The Study

30 women diagnosed with PCOS according to the National Institutes of Health 1990 criteria* were enrolled into the study. The study was designed as a randomised, controlled feeding, crossover trial, testing the effects of two diets differing in glycaemic load [GL]:

- Low GL Diet: 41% carbohydrate, 19% protein, 40% fat
- High GL Diet: 55% carbohydrate, 18% protein, 27% fat

Participants were randomised to start with one of the two diets, which was followed for 4-weeks. This was followed by a 4-week washout period, after which participants crossed over to the other diet [i.e., whichever diet they did not begin the study with] for a further 4-weeks.

Before and after each diet phase, participants underwent various tests, including a meal test with either a high GL or low GL meal. Following the test meal, blood samples were taken over 4hrs. The study investigated the differences in the responses of ghrelin, glucagon, glucose, insulin, and GLP-1, as well as subjective hunger scores, before and after each diet phase.

*Geek Box: The PCOS Diagnostic Criteria Tug-of-War

You may recall from previous Deepdives that we discussed what is colloquially known as the "Rotterdam criteria", which reflected the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine [ESHRE/ASRM] report from 2003, and included:

i. High androgens with clinical oligo/anovulation [i.e., irregular/infrequent (oligo) or absence of (ano) menstrual cycles]

i. High androgens with polycystic ovaries present on ultrasound, but with ovulatory cycles

ii. Clinical anovulation with polycystic ovaries on ultrasound, but without high androgens

iii. High androgens, clinical anovulation, and polycystic ovaries on ultrasound

The initial diagnostic criteria set out in 1990 by the National Institute of Health [NIH] included:

ii. High androgens

iii. Oligoovulation

iv. Exclusion of known disorders

*Compared to the 1990 NIH criteria, what the Rotterdam criteria in effect added was two new PCOS phenotypes: one defined by women with ovulatory cycles but showing polycystic ovaries combined with elevated androgens, and the other defined by women with oligo/anovulatory cycles and polycystic ovaries but **without** elevated androgens.*

Some have argued that the introduction of these additional phenotypes was premature. Others have argued that the complexity of PCOS requires a more expansive diagnostic criterion. The heterogeneity of PCOS presentations arguably lends more weight to the latter argument.

Results: women completed the study, with an average of 31yrs, of which 38% of participants were White/Caucasian and 62% were Black and Hispanic.

- ***Hormonal Outcomes*:** There was a significant difference in postprandial ghrelin levels comparing the two diets, which was higher following the High-GL diet; this effect was most pronounced 3-4hrs after the test meal. Glucagon was significantly higher in the Low-GL group, which was both higher at time 0 [i.e., before the test meal] and from 1-4hrs after the test meal. There were no significant differences between diets on glucose, insulin, GLP-1, or PYY.

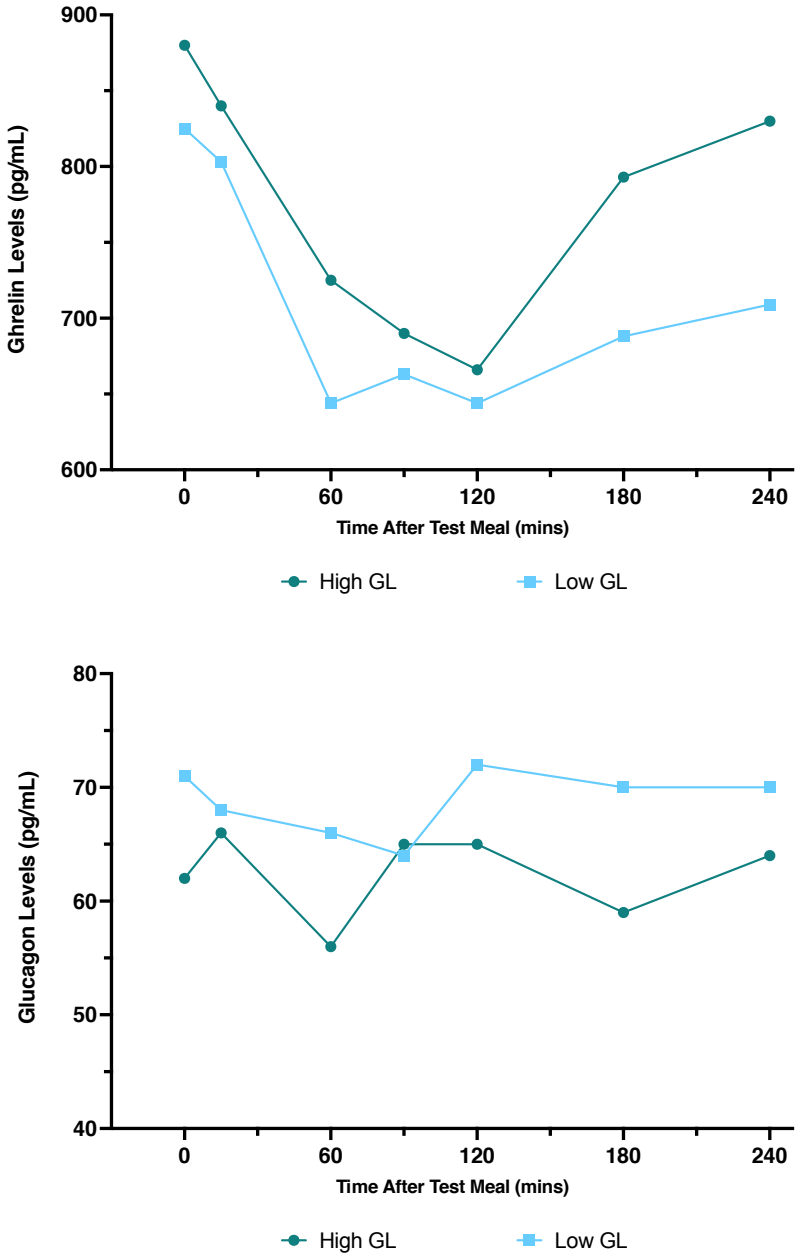

*Figure illustrating [**top**] the differences in ghrelin between the High-GL [**green line**] and Low-GL [**blue line**] groups, and [**bottom**] the differences in glucagon between groups, over 240min postprandial following the test meals.*

- ***Subjective Hunger/Appetite*:** There were no significant differences between diets in relation to subjective hunger, desire to eat, or fullness.

The Critical Breakdown

Pros: The design was good, with the order of diet randomised, an adequate washout period between diets to ensure no carryover effects from the preceding diet, and all foods provided to participants throughout the study. Participants were blind to the order of diet. Diets were designed to maintain bodyweight, and individual energy requirements were determined based on baseline laboratory measurements of energy expenditure. Although the macronutrient profiles of the diets differed, protein intake was matched between groups. The participant group was more ethnically diverse than many studies in this area, with 62% Black and Hispanic participants.

Cons: While the diets were matched for energy intake, the difference in macronutrient composition lets the strength of the design down. GL is a characteristic of carbohydrate composition, not necessarily total carbohydrate intake. If GL was really the exposure of interest, isolating the effects of GL would have been better served by matching energy *and* macronutrients between diets, only manipulating the *type* of carbohydrates in each diet to alter the GL of the respective diets. We're not angry, we're just disappointed. Participants did lose weight, but these data were not presented in the study. As a secondary analysis, the study is limited in drawing causal conclusions [more under ***Key Characteristic***, below].

Key Characteristic

Reading a study that was performed as a secondary analysis can often feel like reading the primary intervention, so it is important to distinguish between the primary intervention and a secondary analysis. A secondary analysis, also known as a 'post-hoc' analysis, is an analysis which is undertaken after a trial has concluded, using data from that trial, to look at a question that was not pre-specified.

Thus, while all data analysis is conducted after a study is concluded, the key feature of a post-hoc analysis is that it is secondary to the study, and the question it is addressing was not a primary research question. In the present study, the primary research question was the effects of the two GL diets on androgens, insulin, and beta-cell function. Thus, the study was not necessarily designed to examine ghrelin and glucagon.

However, where the researchers have taken blood samples, those samples lie in the minus 80 freezers waiting for some years. There may be more analyses which could be conducted from those samples, and research is expensive; why let good samples go to waste? Thus, for the present study, a further analysis was conducted examining differences in outcomes like ghrelin and glucagon.

However, it is important to distinguish that because it is a secondary analysis and not part of the pre-planned study, in effect a post-hoc secondary analysis is an observational study. It can look at relationships, but not causality. Post-hoc analyses are very useful, and informative, tools in research, particularly where a well-conducted controlled trial provides substantial amounts of data.

Interesting Finding

Note that at baseline before the test meals, glucagon was higher in the Low-GI group, and largely remained higher throughout the postprandial period. On the other hand, ghrelin was lower in the Low-GI group. An analysis was conducted to determine how glucagon and ghrelin correlated, and showed that there was a very strong correlation [r = -.81] between glucagon and ghrelin.

Recall that r is a measure of strength of correlation ranging from -1 to 1; -1 is a perfect negative correlation, 0 is no correlation at all, and 1 is a perfect positive correlation. Thus, as you can see from the r of -.81, this is a very strong correlation. You'll also note it is a *negative* correlation, which indicates that *higher* glucagon levels correlated with *lower* ghrelin levels.

Now, recall that glucagon is the *counterregulatory* hormone to insulin, i.e., while insulin helps lower blood glucose levels to normal ranges *after* meals, glucagon helps to regulate glucose levels in the fasted state. However, glucagon is also released briefly in the immediate period following the start of a meal, and appears to have a role in the regulation of satiety and termination of eating [(6)]. The fact that there was such a correlation between glucagon and ghrelin indicates a potential synergistic effect in the regulation of satiety, which would warrant further investigation.

Relevance

First, let us remind ourselves that as a secondary analysis of an RCT, the present study is effectively an observational study. But let' s also remind ourselves that observational research is incredibly valuable, and this study does yield some important data.

The first and most obvious is the main finding that the Low-GI diet resulted in significantly lower ghrelin levels. In the first instance, this warrants comment because previous research has suggested that diet composition has no effect on ghrelin in women with PCOS [(7)]. However, although that research had similar levels of carbohydrates in the comparison diets as the present study, it was primarily comparing a 30% protein vs. 15% protein diet [(7)]. The fact that the present study matched protein between groups at a higher ~18-19% may mean that the associations with ghrelin are more a reflection of the carbohydrate content and GL, given that dietary fat has little impact on ghrelin [(8)].

Does this mean a Low-GI diet has a positive impact on hunger and satiety in women with PCOS? Maybe not. Recall that there were no significant differences in subjective hunger/appetite between groups. Previous research has shown that, independent of ghrelin levels, women with PCOS experience an earlier return to hunger following a meal [4]. And it is also important to note that the satiating effect of high protein diets is *not* due to effects on ghrelin [protein influences other satiety mechanisms] [9].

All of this is to say that the functional relevance of ghrelin, and the influence of diet, in PCOS remains to established. However, if the reduction in ghrelin with Low-GL diets is shown in a direct intervention testing this question, it may be another benefit to manipulating carbohydrate type in PCOS.

Application to Practice

It is important to note that in the primary intervention from which this secondary analysis was conducted, the Low-GL diets resulted in preferential loss of abdominal fat mass and improvements in insulin response and insulin sensitivity, which corresponded to reductions in circulating testosterone levels [10,11]. Indeed, the combination of higher [up to 30% energy] protein *and* low GL carbohydrate [in the context of <50% total energy from carbs] appears to consistently improve androgens and insulin sensitivity [12]. Whether there is a genuine effect on ghrelin and postprandial hunger/appetite regulation is still an open question, but there is sufficient wider evidence to endorse this dietary manipulation regarding protein and GL in women with PCOS.

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