ALINEA

NUTRITION

DECEMBER 2022

www.alineanutrition.com

TABLE OF

CONTENTS

What We Know, Think We Know, or Are Starting to Know 03

The Study 04

Geek Box: Use of Visual Analogue Scales in Nutrition Research 04

Results 05

The Critical Breakdown 06

Key Characteristic 06

Interesting Finding 07

Relevance 07

Application to Practice 08

References 09

Japur CC, Diez-Garcia RW, de Oliveira Penaforte FR, das Graças Pena G, de Araújo LB, de Sá MFS. Insulin, ghrelin and early return of hunger in women with obesity and polycystic ovary syndrome. *Physiol Behav*. 2019;206:252-258.

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

In a previous Deepdive, we looked into an intervention trial that showed higher levels of the "hunger hormone", ghrelin, in women with Polycystic Ovary Syndrome [PCOS] following a high glycaemic load [GL] meal compared to a low GL meal.

We highlighted that although previous research is inconsistent in relation to effects of diet composition on ghrelin levels in women with PCOS, women with PCOS nevertheless have lower levels of gastrointestinal appetite-regulating hormones in response to food intake [(1)]. Ordinarily, ghrelin levels rise prior to feeding but are suppressed in the postprandial period resulting in a decrease in appetite [(2)].

Beyond the role of ghrelin in appetite regulation, ghrelin does not exist in a vacuum in the complex hormonal landscape of PCOS. There are ghrelin receptors in the pancreas, and ghrelin plays a role in maintaining blood glucose homeostasis [(3)]. Experimental research has shown that elevated ghrelin levels inhibit insulin secretion from the pancreas in response to rises in blood glucose levels [(3)].

Now, with your PCOS hat on, you're now thinking… "but isn't PCOS characterised by *high* insulin levels?" And you would be quite correct that most PCOS phenotypes are characterised by high insulin levels [(4)]. Some experimental research has suggested that in women with PCOS, improving insulin sensitivity may enhance appetite regulation mediated by ghrelin [(5)]. Yet, despite some elegant mechanistic work on this topic, no research had – until the present study – examined the associations between pre and post-meal ghrelin and insulin levels, subjective hunger, and energy intake in response to a test meal.

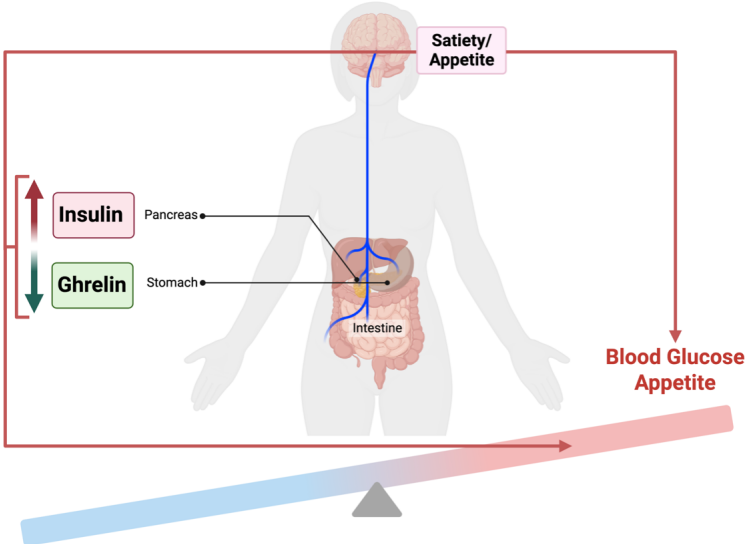

***Figure** illustrating the potential interaction between ghrelin and insulin in PCOS. Decreased ghrelin may correlate with elevated insulin, in turn leading to elevated blood glucose levels and impaired glucose tolerance. Low ghrelin may also feedback to brain appetite circuits, lowering satiety and increasing hunger levels, or resulting in earlier return to hunger following a meal.*

The Study

The study recruited 53 women for a cross-sectional analysis of women with PCOS [*n* = 30] and non-PCOS women for comparison [*n* = 23]. The aim of the study was to compare the associations between insulin, ghrelin, and subjective hunger in response to an *ad libitum* [Latin for "at one's pleasure", this is where participants are free to eat as much as desired] test meal.

Participants ate a fixed standardised meal [300kcal; 73% carbohydrate] at 9am, before the *ad libitum* test meal was served at 12pm. Before the test meal, participants had blood samples drawn to assess insulin, glucose, ghrelin, testosterone, and also completed a visual analogue scale* [VAS; for more detail on this method, see ***Geek Box**, below].

Following the meal, further blood draws and VAS assessments at 15min, 30min, 60min, and 120min in the postprandial period were taken. The analysis compared the hunger and metabolic measures between groups and between the respective postprandial times.

*Geek Box: Use of Visual Analogue Scales in Nutrition Research

Visual analogue scales [VAS] are common psychometric tools in both psychology and nutrition research, used to measure a particular preference, attitude, or characteristic that may have a range of potential values. For example, hunger and fullness are both subjective, and may differ across a range of responses depending on time of day, fed or fasted state, time since last meal, etc.

The simple form of VAS used is a straight, horizontal line, commonly around 100mm [10cm] in length. The far left will generally represent the lowest end of the variable being measured and each end of the line corresponds to a verbal anchor, i.e., with fullness 0mm could be 'not hungry at all' while 100mm could be 'extremely hungry'. Participants are asked to make a vertical line with a pen/pencil/marker across the horizontal measurement line, at a point which represents for them how they feel in response to that question.

This is then turned into data the old-fashioned way: by getting out a ruler, and measuring the point at which the participant made their line crossing the horizontal measurement line. For example, on a 100mm [10cm] hunger scale it could be 70.6mm [7.6cm] - this is therefore the data point for that time. Imagine that was pre-meal, and then you measure the participant again 30mins later: now it could be 20.3mm [2.3cm], indicating a shift to a more satiated state following the meal, i.e., a decrease in hunger.

VAS can be useful for certain measures that exist on a subjective continuum, rather than using, for example, Likert scales where participants could have a 1-5 score of pre-defined values, e.g., 'mild', 'moderate', 'severe'. In this regard, VAS can be more sensitive to smaller, incremental changes in a particular measure than categorical scales They are also very simple to use and time-efficient for research purposes. They do have certain disadvantages or things to consider, however.

They are primarily subjective, which is not a limitation per se, but an important point to bear in mind when the outcome is a variable that could also have physiological measures. They are also validated in very specific contexts, which may not always be generalisable to every circumstance in which they are used. So, it is important to think about the validation of a particular VAS, the context in which that validation occurred, and how that context relates to its use in another study.

Results: Average age of participants was 29yrs and 33yrs in the PCOS and non-PCOS groups, respectively. Average BMI was 35.3kg/m^2 and 34.6kg/m^2 in the PCOS and non-PCOS groups, respectively.

- ***Pre-Meal Insulin, Ghrelin and Hunger Levels*:** At baseline, insulin levels were significantly higher in the PCOS compared to non-PCOS group [21.6uIU/mL vs. 12.6uIU/mL ('normal' is <25uIU/mL)].

Ghrelin levels were lower in the PCOS group [571.4pg/mL vs. 595.0pg/mL], while subjective hunger was slightly higher [61.0/100 vs. 57.5/100] in the PCOS group. In the PCOS group, pre-meal ghrelin showed a moderate correlation with hunger levels, which was not shown in the control group.

- ***Ad Libitum Energy Intake*:** Both groups consumed similar *ad libitum* energy intake during the test meal, with the PCOS and non-PCOS groups consuming 606kcal and 557kcal, respectively. In women with PCOS who exhibited insulin resistance, energy intake was 759kcal compared to 565kcal in PCOS women without insulin resistance.
- ***Post-Meal Insulin, Ghrelin and Hunger Levels*:** Over the 2 h postprandial period, compared to the non-PCOS group, insulin levels were 36% higher while ghrelin levels were 8% lower and blood glucose levels 5% lower, in the PCOS group.

Hunger levels were significantly higher across the 2 h postprandial period in the PCOS group compared to the non-PCOS group [see ***figure***, below].

Women with PCOS showed a significant increase in hunger 60min post-meal, compared to 120min post-meal in the non-PCOS group [more under ***Interesting Finding***, below].

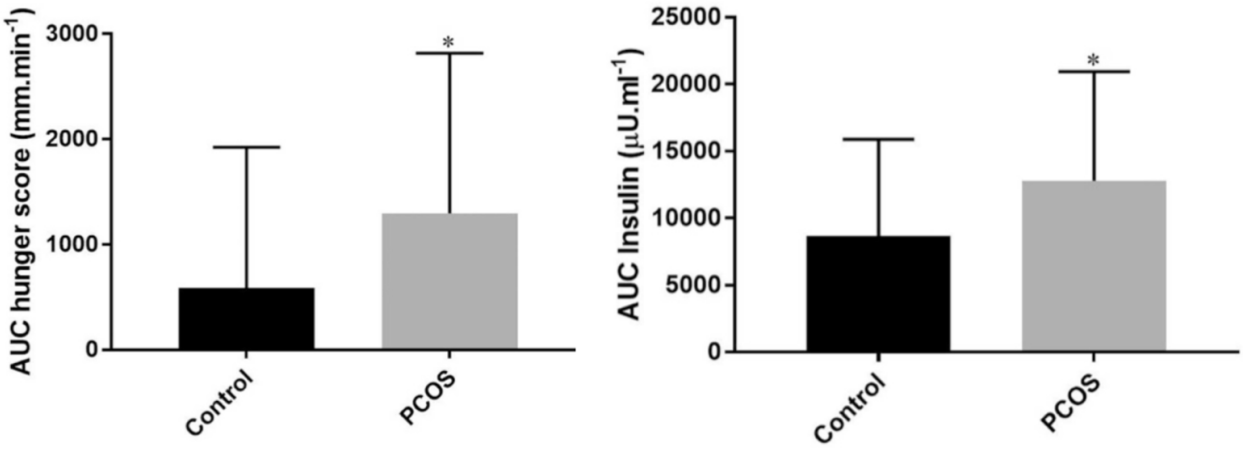

***Figure** from the paper illustrating the differences between PCOS group [**grey bars**] and non-PCOS controls [**black bars**] in relation to hunger [**left**] and insulin [**right**] over 2 h following the ad libitum test meal. Despite similar levels of energy intake consumed between PCOS and non-PCOS controls, women with PCOS exhibited higher postprandial insulin levels, higher hunger levels, and earlier return to hunger, compared to non-PCOS controls.*

The Critical Breakdown

Pros: The study had clearly stated aims and hypothesis. Women were diagnosed with PCOS according to the defined Rotterdam Criteria. The provision of a standardised breakfast to all participants before the *ad libitum* test meal, may have helped to balance hunger levels between groups before the test meal. The test meal itself was *ad libitum*, which allowed for a more "real world" test of how ghrelin, insulin, and appetite, may interact to influence energy intake in women with PCOS. The study appears to have met its required sample size estimate for adequate statistical power.

Cons: The study was a non-randomised, cross-sectional study where participants were divided according to PCOS diagnosis or non-PCOS controls. Given that the investigators still implemented an intervention with the experimental test meal, it seems like a missed opportunity to have increased the methodological rigour of the trial by availing of randomisation. The primary outcomes are not clearly stated, although we can infer that they were ghrelin and insulin. The postprandial analysis was confined to a 2 h period from a single test meal, so caution is required against over-extrapolating the findings to effects over an entire day. There were more women in the PCOS group [$n = 30$] compared to the non-PCOS group [$n = 23$], which may have introduced some bias towards the PCOS group. The results section also begins to discuss a subgroup analysis of participants with insulin resistance, however, this [or any] subgroup analysis is not mentioned in the methods section.

Key Characteristic

This study appears to have been the first to consider both objective appetite regulatory hormones, i.e., ghrelin, and subjective hunger scores assessed in the postprandial period. And this provides some important insights, limitations of the study notwithstanding, given that there appeared to be little correlation between either ghrelin or insulin and postprandial hunger levels.

Recall that correlations are represented as "*r*", where r is a measure of the 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. For ghrelin, the correlation was $r = .30$, which would be a weak positive correlation. This finding is in fact consistent with previous research that has considered ghrelin, which has shown no clear evidence that lower ghrelin levels correlate to any strong degree with subjective hunger, appetite, or desire to eat, in women with PCOS [(1)].

This is why the ***Key Characteristic*** of the present study is that it assessed subjective hunger, which leads us to our ***Interesting Finding*** from this paper…

Interesting Finding

The main finding of the present study is also its most interesting result, namely the earlier return to hunger in the PCOS group compared to the non-PCOS group. At 15min and 30min postprandial, hunger levels were similar between groups following the *ad libitum* meal. However, by 60min postprandial the PCOS group hunger levels were significantly higher compared to the 15min postprandial measure, while in the non-PCOS control subjective hunger was only significantly different to the 15min measure by the final measurement at the 2 h postprandial mark.

Given the lack of any strong correlations between ghrelin or insulin and subjective hunger, the inference from this finding is that women with PCOS exhibited an earlier return to hunger independent of ghrelin and insulin. And, importantly, because there was only a negligible difference in energy consumed during the *ad libitum* test meal, the inference from this finding is that the earlier return to hunger in women with PCOS compared to non-PCOS controls may be evident despite similar levels of energy intake. All of which provides some support to the inconsistent evidence that suggests women with PCOS exhibit some degree of appetite dysregulation [(6)].

Relevance

The pathophysiology of PCOS is so complex that the idea that there will be a simple correlation that explains certain of the interactions with hunger, appetite, and energy intake, is likely to be wishful thinking. And overall, the body of evidence is limited by small trials with an array of findings, often not very robust in their methodological design.

Nevertheless, there are some pieces to the overall pathophysiological PCOS puzzle that we may try to link together. The first piece of the puzzle is that it does appear to be a reliable finding that women with PCOS have lower ghrelin levels compared to non-PCOS controls, with the most recent meta-analysis on this question supporting this finding [(7)].

The second piece of the puzzle is that previous research has also demonstrated a relationship between ghrelin and insulin resistance in PCOS, i.e., that low ghrelin levels correlate with insulin resistance [(8)]. However, it is possible that a strong correlation between ghrelin and insulin resistance may only be present in individuals with severe insulin resistance [(9)].

This latter point may have relevance for the findings in the present study, where most participants with PCOS were not severely insulin resistant, and a previous publication from this study showed no significant correlation between insulin and ghrelin in the women with PCOS [(10)].

Finally, what of the last piece of the puzzle from this study: hunger and appetite. The present study suggests that, independent of ghrelin and with a similar level of energy intake compared to non-PCOS controls, women with PCOS are hungrier, earlier after consuming a meal. This is not an isolated finding; a more well executed, randomised trial showed that, compared to women without PCOS, women with PCOS were less satiated and hungrier after eating [(1)].

Perhaps the lower satiety and higher hunger levels in women with PCOS are independent of ghrelin, however, better designed, and larger trials will be required to get a more definitive answer on this question.

Application to Practice

Is it possible to “use the evidence that is there” rather than simply say “we need more evidence”?

If we assume that the finding that women with PCOS do exhibit greater hunger and less satiety is a real effect, there is evidence that higher protein, moderate carbohydrate intakes, and frontloading energy intake in the early period of the day, may improve appetite [11] while lowering insulin and androgen levels in women with PCOS [12,13].

This may be a prudent approach to diet, emphasising the combination of higher – up to 30% energy – protein intakes, in combination and moderate [~40-45%] carbohydrate intakes, and low GL carbohydrate sources.

References

1. Moran LJ, Noakes M, Clifton PM, Wittert GA, Tomlinson L, Galletly C, et al. Ghrelin and Measures of Satiety Are Altered in Polycystic Ovary Syndrome But Not Differentially Affected by Diet Composition. J Clin Endocrinol Metab. 2004 Jul;89(7):3337–44.
2. Cummings DE, Weigle DS. A Preprandial Rise in Plasma Ghrelin Levels Suggests a Role in Meal Initiation in Humans. Diabetes. 2001;50:1714–9.
3. Tong J, Prigeon RL, Davis HW, Bidlingmaier M, Kahn SE, Cummings DE, et al. Ghrelin Suppresses Glucose-Stimulated Insulin Secretion and Deteriorates Glucose Tolerance in Healthy Humans. Diabetes. 2010 Sep 1;59(9):2145–51.
4. Rojas J, Chávez M, Olivar L, Rojas M, Morillo J, Mejías J, et al. Polycystic Ovary Syndrome, Insulin Resistance, and Obesity: Navigating the Pathophysiologic Labyrinth. Int J Reprod Med. 2014;2014:1–17.
5. Romualdi D, de Marinis L, Campagna G, Proto C, Lanzone A, Guido M. Alteration of ghrelin-neuropeptide Y network in obese patients with polycystic ovary syndrome: role of hyperinsulinism. Clin Endocrinol (Oxf). 2008 Oct;69(4):562–7.
6. Ee C, Pirotta S, Mousa A, Moran L, Lim S. Providing lifestyle advice to women with PCOS: an overview of practical issues affecting success. BMC Endocr Disord. 2021 Dec 23;21(1):234.
7. Gao T, Wu L, Chang F, Cao G. Low circulating ghrelin levels in women with polycystic ovary syndrome: a systematic review and meta-analysis. Endocr J. 2016;63(1):93–100.
8. 8. Pagotto U, Gambineri A, Vicennati V, Heiman ML, Tschöp M, Pasquali R. Plasma Ghrelin, Obesity, and the Polycystic Ovary Syndrome: Correlation with Insulin Resistance and Androgen Levels. J Clin Endocrinol Metab. 2002 Dec;87(12):5625–9.
9. Tschöp M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating Ghrelin Levels Are Decreased in Human Obesity. Diabetes. 2001 Apr 1;50(4):707–9.
10. Japur CC, Diez-Garcia RW, de Oliveira Penaforte FR, de Sá MFS. Imbalance Between Postprandial Ghrelin and Insulin Responses to an Ad Libitum Meal in Obese Women With Polycystic Ovary Syndrome. Reproductive Sciences. 2014 Aug 1;21(8):1020–6.
11. Jakubowicz D, Froy O, Wainstein J, Boaz M. Meal timing and composition influence ghrelin levels, appetite scores and weight loss maintenance in overweight and obese adults. Steroids. 2012;77(4):323–31.
12. Jakubowicz D, Barnea M, Wainstein J, Froy O. Effects of caloric intake timing on insulin resistance and hyperandrogenism in lean women with polycystic ovary syndrome. Clin Sci. 2013;125(9):423–32.
13. Mehrabani HH, Salehpour S, Amiri Z, Farahani SJ, Meyer BJ, Tahbaz F. Beneficial Effects of a High-Protein, Low-Glycemic-Load Hypocaloric Diet in Overweight and Obese Women with Polycystic Ovary Syndrome: A Randomized Controlled Intervention Study. J Am Coll Nutr. 2012 Apr;31(2):117-125.