Carbohydrate Intake Prior to Oral Glucose Tolerance Testing

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Abbreviations: ADA, American Diabetes Association; HbA1c, glycated hemoglobin A1c; IGT, impaired glucose tolerance; LC-HF, low-carbohydrate high-fat; OGTT, oral glucose tolerance test; PG, plasma glucose; WHO, World Health Organization

Abstract

With the emergence of glycated hemoglobin as a diagnostic test for diabetes, oral glucose tolerance tests (OGTTs) have become rare in endocrinology practice. As they have moved out of favor, the importance of patient instructions on preparation prior to OGTT has faded from memory. Decades-old literature, well-known to endocrinologists a generation ago, emphasized the importance of carbohydrate intake prior to OGTT. In this expert endocrine consult, we discuss an OGTT performed in a research setting without adequate carbohydrate intake at the evening meal prior to the OGTT. The resultant elevated plasma glucose levels at 1-hour and 2-hours mimicked the loss of first-phase insulin release seen in early type 1 and type 2 diabetes. With clinical concern that the research participant had evolving type 1 or type 2 diabetes, the volunteer was subjected to additional testing and experienced anxiety. Repeat OGTT was normal after adequate carbohydrate intake (>150 grams/day and >50 grams the evening prior to overnight fast for the study). The physiology of this phenomenon is explored and is likely mediated through beta cell adaptation and alteration in peripheral glucose uptake in response to nutrient exposure. The learnings of decades ago have clearly faded, and this literature should be revisited to ensure that OGTT results are not compromised when ordered for clinical or research purposes.

Key Words: oral glucose tolerance test, low carbohydrate, diabetes, impaired glucose tolerance

The acceptance of glycated hemoglobin (HbA1c) for the diagnosis of diabetes and prediabetes has reduced the number of oral glucose tolerance tests (OGTTs) being performed in nonpregnant adults. With its limited use, the importance of proper preparation prior to administration of an OGTT, well-established long ago, has faded from the literature and guidelines. In this expert endocrine consult, we report a false-positive OGTT in the setting of a single low-carbohydrate meal prior to testing and underscore a literature that has been forgotten over the past generation but still has important implications in both clinical care and research.

Case Report

A 20-year-old healthy woman volunteered for a research study that required patients with no history of diabetes to undergo an OGTT in order to evaluate the effect of circulating glucose on neuronal excitability. A physically active college student, the volunteer had a body mass index of 20.7 kg/m^2 and no personal or family history of diabetes. At screening, her fasting plasma glucose (PG) was 71 mg/dL. She qualified for the study and was instructed to fast for 12 hours prior to presentation. At the study visit, fasting PG was 60 mg/dL. A 75-gram OGTT was performed in a research setting by experienced staff. One hour post–glucose load, her PG was 153 mg/dL (Table 1). Two hours post–glucose load, her PG was 200 mg/dL (Table 1).

Given concern that this test result indicated the diagnosis of diabetes, a medical history and extensive dietary recall was obtained. The day prior to the abnormal OGTT, she reported eating a high-carbohydrate breakfast and lunch from a fast-food restaurant that included French fries. Dinner consisted of eggs, turkey bacon, avocado on toast, and 8 ounces of orange juice (less than 50 grams net carbohydrates). She confirmed that she had no risk factors for type 2 diabetes, no history of disordered eating, and no family or personal history of autoimmunity. She acknowledged anxiety about the potential diagnosis of diabetes.

Two national diabetes experts with a focus on type 1 diabetes prevention and management were presented the findings and decades-old literature that suggest that a low-carbohydrate diet (<150 grams/day and <50 grams prior to fasting for the test) can lead to false-positive OGTTs. Both experts acknowledged the literature regarding carbohydrate loading prior to OGTT, but suggested that the presentation was consistent with early (stage 2) type 1 diabetes [1]. Each recommended antibody testing as well as a repeat OGTT after appropriate and documented dietary preparation.

One week after she was informed of the abnormal OGTT, the patient repeated a 75-gram OGTT in a primary

Table 1. OGTT results

	Experimental protocol		Clinical exam
	Screening	OGTT (low-carb dinner)	OGTT (high-carb diet)
Time (min)	Glucose (mg/dL)		
0	71	60	71
30	-	153	-
60	-	153	-
120	-	200	75
180	-	152	-

During the experimental protocol, single pulse transcranial magnetic stimulation and a working memory task was performed while high-density electroencephalography was recorded at each OGTT time point. The first OGTT was performed after a low-carbohydrate dinner prior to a 12-hour fast before the study. The clinical exam OGTT was performed in a primary care setting after 3 days of >150 g/day carbohydrate ingestion and specifically a high-carbohydrate meal the night prior to the OGTT.
Abbreviations: carb, carbohydrate; OGTT, oral glucose tolerance test.

care setting. She was instructed to eat a minimum of 150 grams carbohydrates for 3 days prior to testing. She completed a food log that was confirmed to document >50 grams carbohydrate per meal by her physician. Blood was collected for HbA1c and GAD65 antibodies. Repeat OGTT revealed a fasting PG of 71 mg/dL (Table 1). Two hours after a 75-gram glucose load, PG was 75 mg/dL (Table 1). Her HbA1c was 5.0% (31 mmol/mol). GAD65 antibodies were negative. The patient met no criteria for diabetes or prediabetes on repeat testing.

Discussion

"Hunger diabetes" was first described in the late eighteenth century after the discovery of glucosuria in starving dogs fed a high-carbohydrate meal [2]. Fifty years later, the clinical importance of diet prior to OGTT was described. Well- and undernourished individuals who had normal glucose tolerance after a 5-day 300 gram carbohydrate preparatory diet were restricted to 20 grams carbohydrate for 5 days [2]. Restriction of carbohydrates resulted in delayed clearance of absorbed glucose in all individuals and was exaggerated in undernourished participants. After low-carbohydrate diets, all undernourished individuals met criteria for glucose intolerance or diabetes. Dr. Conn thus recommended a standard 300-gram carbohydrate diet prior to OGTT [2].

Wilkerson et al challenged that a 150-gram carbohydrate diet is sufficient in most normally nourished individuals. Restriction of carbohydrates to 20 grams for 5 days resulted in impaired glucose tolerance (IGT) in males and females [3]. Twenty-two percent of women (2

out of 9) and no men met criteria for diabetes, suggesting that sex differences may exist in glucose tolerance in response to carbohydrate restriction [3]. Based on these data, the World Health Organization (WHO) has long recommended preparation for OGTT with greater than 150 grams carbohydrate per day [4]. This recommendation, buried in the appendix of the 1985 WHO report, has been minimized further in updates. The current American Diabetes Association (ADA) Standards of Care cite the WHO report, but the document does not explicitly state the need for dietary guidance prior to OGTT [1]. Similarly, the American Association of Clinical Endocrinologists and American College of Endocrinology (AACE/ACE) guidelines for the diagnosis of diabetes do not mention the necessary carbohydrate loading prior to testing [5]. Notably, the 2008 ACE/AACE guidelines for the diagnosis and management of prediabetes indicates the necessity of adequate carbohydrate intake prior to OGTT but does not define the amount of carbohydrate required [6].

More recent data, although still more than 20 years old, demonstrated that some individuals who ate a normal carbohydrate breakfast and lunch (60% carbohydrate, >75 grams) followed by a low-carbohydrate dinner (10% carbohydrate, <50 grams) exhibited IGT, even when the daily carbohydrate totaled greater than 150 grams [7]. In this study, all participants (8 men and 4 women) had normal fasting glucose irrespective of their carbohydrate intake. All participants had normal glucose tolerance the morning after a single high-carbohydrate meal (80% carbohydrate, >100 grams). However, despite a daily total of ≥150 grams carbohydrate, 38% of men (3 out of 8) and 25% of women (1 out of 4) demonstrated IGT after a single low-carbohydrate meal (<50 grams) immediately prior to testing. These data are consistent with the case presented here, in which the patient ate greater than 150 grams carbohydrates daily, but limited carbohydrates in her final meal prior to the abnormal OGTT. We are not aware of any guideline that explicitly states the necessity of a normal- to high-carbohydrate meal (50 grams minimum) immediately prior to the fasting period before presentation for the OGTT.

OGTTs are ordered less frequently by clinical endocrinologists. Conversely, obstetricians order OGTTs on nearly all pregnant nondiabetic patients. While some studies suggest that carbohydrate loading does not affect the OGTT in pregnancy [8-10], none of these studies compare confirmed low-carbohydrate diets (less than 150 grams/day) with higher carbohydrate diets. Moreover, similar to the case presented here, a very-low-carbohydrate meal (6.7% carbohydrate, <10 grams) immediately prior to OGTT has been shown to alter OGTT results during pregnancy [11]. Recent data also suggest that low-carbohydrate diets

are associated with positive 1-hour OGTT and diagnosis of gestational diabetes in pregnancy diabetes screening programs [12, 13]. As gestational diabetes diagnosis is increasing, some of the obstetrics community recognizes the flawed nature of OGTT and posits that alternative screening tools will eventually replace the OGTT for detection of gestational diabetes [14]. Until then, more research to understand how carbohydrate intake impacts OGTT in pregnancy is warranted and a standardized protocol for OGTT preparation is essential.

The mechanisms of how low-carbohydrate diets impact glucose metabolism are complex and incompletely understood. Some propose that the mechanism is in part due to loss of first-phase insulin release resulting in decreased peripheral and hepatic glucose uptake and incomplete suppression of hepatic glucose production [15-17]. Loss of first-phase insulin is well characterized in both type 1 and type 2 diabetes and occurs early in the evolution of diabetes [18, 19]. Investigators in the Diabetes Prevention Trial of Type 1 Diabetes (DPT-1) identified a subgroup of individuals with islet cell antibodies and impaired first-phase insulin response who were asymptomatic and had normal fasting PG, but who were diagnosed with diabetes via OGTT [20]. Loss of first-phase insulin release is also well documented in type 2 diabetes, although whether it precedes insulin resistance has been debated [19]. Hyperglycemic clamp experiments in individuals with IGT demonstrate loss of first- and second-phase insulin release [19, 21]. First-phase insulin release is reduced to a greater degree than second-phase insulin release, suggesting that the primary defect may start with first-phase insulin loss. Insulin sensitivity was also decreased in individuals with IGT, but to a lesser extent, suggesting that type 2 diabetes is defined first by beta cell dysfunction, rather than insulin resistance [19]. Indeed, seminal work led by John Gerich demonstrated that subjects with normal glucose tolerance and a first-degree relative with type 2 diabetes have decreased first- and second-phase insulin release without a defect in insulin sensitivity [19, 22]. Although supportive data is limited, as no insulin levels were drawn during the study, the OGTT presented here after a single low-carbohydrate meal appears to mimic first-phase insulin loss, suggesting that low-carbohydrate intake may impact beta cell function.

Few mechanistic studies employ single low-carbohydrate meals to explore the impact of carbohydrate intake on glucose metabolism. Data from longer-term low-carbohydrate or ketogenic diets therefore provide insight. Animal models have shown that longer-term low-carbohydrate diets result in decreased beta cell mass [23] that is likely reversible [24, 25]. Rats that are fed low-carbohydrate high-fat (LC-HF) or ketogenic diets in which 5% or less of total calories

are derived from carbohydrates for 3 to 8 weeks demonstrate a significant increase in glucose levels during OGTT [23, 25]. Caloric restriction of LC-HF diets to 80% of the isoenergetic pair-fed groups eliminated differences in fat mass, but glucose intolerance was maintained in LC-HF animals, suggesting that adiposity was not the driver of abnormal glucose metabolism [23]. Instead, total pancreas volume and total beta cell volume was significantly lower in rats fed a LC-HF diet compared with controls [23]. The cause of this loss of beta cell mass is multifactorial, but loss of insulin- and hyperglycemia-mediated pro-proliferative and hypertrophic effects likely contribute [26–29]. Unlike the primary beta cell defect observed in type 1 and type 2 diabetes, studies have demonstrated that the beta cell dysfunction caused by low-carbohydrate diets are likely reversible, as resumption of normal carbohydrate diet restores glucose homeostasis [25].

Low carbohydrate intake also impacts insulin sensitivity in both animal models and humans. Euglycemic clamp studies in rats and mice fed ketogenic diet for 3 to 8 weeks demonstrate impaired hepatic and peripheral insulin sensitivity [23, 30]. When fed a ketogenic diet, both rodent models required a significantly lower glucose infusion rate to maintain a euglycemic clamp, indicating whole-body insulin resistance [23, 30]. Additionally, the ability of the glucose infusion to suppress endogenous glucose production was significantly impaired compared to regular chow fed animals, suggesting hepatic-specific insulin resistance [23, 30]. Similar hepatic insulin resistance has been shown in mice following only 3 days of ketogenic diet [31].

In humans, data from highly trained athletes who were subjected to 6 months of LC-HF diets (~50 grams/day carbohydrate) demonstrated high glucose concentrations and delayed peak insulin concentrations during OGTT and reduced expression of key proteins of the insulin signaling pathway, glucose transporter 4 (GLUT4) and insulin receptor substrate 1 (IRS1) [32]. IRS1 phosphorylation initiates the insulin cascade, which is critical for translocation of GLUT4 to the plasma membrane and rapid uptake of glucose in the muscle [32]. Loss of expression of these proteins results in an insulin-resistance-like state, which is unlikely pathological, but instead an adaptation to consistently reduced glucose exposure [32].

Additional mechanisms may also play a role, particularly in acute low-carbohydrate diets. For example, low-carbohydrate diets are associated with an increase in plasma free fatty acids, which may decrease insulin secretion. Fatty acid oxidation is associated with decreased glycolysis, glucose uptake, and glucose oxidation, all of which can ultimately raise PG [16, 24, 33, 34]. Alterations in the level of glucagon-like peptide 1 (GLP-1) have also been observed in association with low-carbohydrate diets (20% of total energy derived from carbohydrates). Short-term low-carbohydrate diets in healthy individuals have been shown to increase the levels of post–glucose load GLP-1, which may be in part to compensate for reduced insulin production [16, 35]. Whether these mechanisms are caused by low-carbohydrate diet or are a result of changes in other metabolic parameters remains to be elucidated.

Nutrition research continues to investigate these mechanisms, particularly in regard to the long-term impact of low-carbohydrate diets on metabolism and clinical disease [36]. These efforts are essential, as ketogenic diets (<50 grams carbohydrate/day) and other lower-carbohydrate meal plans have become increasingly popular. Yet, our understanding of the influence of these diets on the risk of metabolic disease is unknown. Meanwhile, despite a growing number of physicians arguing for increased oral glucose tolerance testing for the identification of dysglycemia [37], many clinicians are forgetting that low-carbohydrate diets may also impact the OGTT as a diagnostic tool.

The utility of HbA1c is unassailable, but like every test, it has flaws [38]. A threshold of HbA1c >6.5% was suggested for the diagnosis of diabetes based on studies demonstrating an association between HbA1c and diabetic retinopathy, but data suggest that this HbA1c level fails to identify a significant population of people with diabetes by OGTT or fasting PG [39, 40]. AACE/ACE and ADA guidelines acknowledge limitations in HbA1c and fasting PG as tools for diagnosis of diabetes, implying that measuring PG after a 75-gram 2-hour OGTT may be the most sensitive test for detecting diabetes [1,5]. Indeed, using the HbA1c cutoff of 6.5% for the diagnosis of diabetes has high specificity (~99%), but the sensitivity is poor (~20%-40%) when compared with the 75-gram OGTT [41]. Moreover, 2-hour PG has a strong association with cardiovascular disease, hypertension, dyslipidemia, and microalbuminuria [41]. Failure to identify individuals with diabetes solely based on positive 2-hour PG risks underdiagnosing and undertreating a population particularly vulnerable to cardiovascular disease.

HbA1c also fails to identify the majority of patients with IGT [42] and early dysglycemia [41], leaving the OGTT as the posited most appropriate tool to identify these patients. Use of OGTT in high-risk patients allows for early intervention and prevention of progression to type 2 diabetes. As such, the ADA specifically recommends OGTT as the preferred method for diagnosis of cystic fibrosis–related diabetes, posttransplantation diabetes mellitus, and in the postpartum period in women with gestational diabetes and notes preferences among some authorities for the diagnosis of diabetes in children [43]. The ADA also notes that PG measurements (fasting PG or 2-hour OGTT) should be used in conditions that alter the relationship of HbA1c and

glycemia, including but not limited to increased red blood cell turnover, HIV treated with certain protease inhibitors, and iron-deficiency anemia [1]. The Endocrine Society also recommends use of OGTT over HbA1c in women with polycystic ovarian syndrome, given the association between IGT and cardiovascular disease in women [44]. Furthermore, emerging data suggest that IGT identified by 2-hour PG provides prognostic information following myocardial infarction, whereas HbA1c and fasting PG do not, paving the way for expanding the use of OGTT to other circumstances [42].

The strength of 1-hour PG >155 mg/dL as an early marker of dysglycemia and predictor of incident diabetes, cardiovascular risk, diabetes complications, and mortality has emerged over the last several years [45-47]. A 1-hour PG >155 mg/dL following a 75-gram OGTT is also more sensitive for detecting individuals at high risk for type 2 diabetes than HbA1c, fasting PG, or 2-hour PG [41]. Individuals with normal glucose tolerance and a 1-hour PG >155mg/dL likely share abnormalities observed in IGT, including beta cell dysfunction and impaired insulin sensitivity [41]. The use of 1-hour OGTT may allow the identification of a vulnerable population that may otherwise be overlooked even by the gold standard of 2-hour PG following a 75-gram OGTT [48]. As 1-hour PG as a diagnostic tool gains traction, clinical OGTT use may increase, and clinicians must be reminded of the testing requirements to permit proper interpretation.

Perhaps most importantly, as in the case presented here, OGTTs are increasingly used in research settings where investigators may not be aware of the importance of proper preparation. Using the search terms *oral glucose tolerance test* or *OGTT* on clinicaltrials.gov on March 11, 2021, yielded >1500 studies that report OGTTs as part of the study protocol. As awareness of appropriate instruction prior to OGTT is lost and investigators less knowledgeable about the issues discussed here employ OGTTs, study outcomes and their volunteers may be impacted. Investigators must therefore be made aware of the activities that can influence test results and contribute to the overall poor reproducibility of the test, especially with regard to the 2-hour post–glucose load value [14, 49-51]. These include patient factors (exercise, stress, sleep, smoking, hydration status, and consumption of caffeine, alcohol, and carbohydrates) and proper collection, storage, and specimen sampling to prevent glucose metabolism following blood draw [14].

Conclusion

The case reported herein, supported by decades-old literature, demonstrates that if the OGTT is administered without full understanding or adherence to the specific protocol of preparation and administration, it can lead to false-positive results, patient distress, and potentially the misdiagnosis of diabetes. More research is necessary to understand why a single low-carbohydrate meal impacts some individuals. In the meantime, as OGTT use increases in an era of low-carbohydrate diets, provider and patient education about dietary preparation for OGTT should be explicit in order to avoid false-positive test results. Guidance should also recommend against smoking, caffeine consumption, and exercise immediately prior to OGTT as they may also impact results [52-54]. Importantly, patients should receive dietary instruction to prepare for the OGTT:

- At least 3 days of "unrestricted diet" (moderate- to high-carbohydrate intake), containing >150 grams of carbohydrates daily
- Ideally, the meal plan should be delivered as 3 daily meals containing at least 50 grams of carbohydrates per meal
- Perhaps most importantly, the last meal the evening before the fasting test should include at least 50 grams of carbohydrates
- Per WHO recommendations, "the test should be preceded by an overnight fast of 10–16 hours during which water may be drunk".

Additional Information

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