OBSERVATIONAL

Cortisol Patterns and DHEA Levels of Patients with Obesity, Prediabetes, and Type 2 Diabetes:

A Chart Review in a Naturopathic Primary Care Clinic

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ABSTRACT

In healthy individuals, cortisol levels exhibit a circadian pattern, peaking in the morning and decreasing the rest of the day. Studies are inconclusive as to the relationship between high total cortisol levels and obesity, prediabetes, and type 2 diabetes. Since cortisol levels are circadian, many naturopathic physicians use a salivary test performed at four time points during the day to measure the overall pattern of secretion, rather than relying upon a blood draw from a single time point. Some physicians hypothesize that a dysregulated pattern of cortisol is more indicative of diabetes risk than a high mean cortisol level. A retrospective chart review was performed on obese, prediabetic, and type 2 diabetes patients in order to test this theory. The goals of this study were to determine whether people with, or at risk for, type 2 diabetes have abnormal circadian cortisol patterns and dehydroepiandrosterone (DHEA) levels. The chart review demonstrated four patterns of cortisol secretion, one of which is circadian, in this population.

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Type 2 diabetes is a multi-factorial disease characterized by mild to severe glucose dysregulation with associations to increased mortality, and the development of polyneuropathy, nephropathy, and heart disease. Diagnoses of obesity and/or prediabetes increases the risk of developing type 2 diabetes.[1,2] In healthy individuals, blood glucose concentrations are maintained between 80 and 100 mg/dL. Ingestion of carbohydrates causes an increase in blood glucose concentration and subsequent release of insulin by beta-islet cells within the pancreas. Insulin lowers blood glucose both by decreasing hepatic and adipose glucose production, and by accelerating the uptake of glucose into peripheral tissues. One of the probable first steps in the development of type 2 diabetes is insulin resistance, defined by impaired sensitivity to a normal concentration of insulin.[3] Insulin resistance is a common factor of obesity, prediabetes, and type 2 diabetes.[4]

In human studies, high cortisol has been shown to contribute to insulin resistance[5] and is likely involved in the development of type 2 diabetes, as well as the persistence of high glucose levels.[6] Cortisol is a glucocorticoid hormone produced by the adrenal cortex that is involved in the regulation of mineralocorticoids, blood pressure, immune function and metabolism.[7] Conditions that involve excess cortisol are hypertension, hypercholesterolemia, central obesity, and glucose intolerance.[8] In fact, one of the likely methods by which cortisol contributes to these diseases is by inducing a state of insulin resistance.[9] As the primary glucocorticoid released during stress, cortisol has a variety of actions: 1) impairs insulin-dependent glucose uptake in the periphery, 2) enhances gluconeogenesis in the liver, and 3) inhibits insulin secretion from pancreatic b-islet cells. All of these actions contribute to elevated glucose levels. Dysregulated cortisol levels have been shown in persons with insulin resistance, prediabetes, and type 2 diabetes.[3,5,6] Prediabetes is characterized by a fasting plasma glucose between 100-126 mg/dL. This is also known as Impaired Fasting Glucose (IFG) or Impaired Glucose Tolerance (IGT). Beyond 126 mg/dL is diagnostic of type 2 diabetes.[10]

Cortisol normally follows a circadian pattern of secretion, peaking 30 minutes after waking followed by a gradual decrease throughout the rest of the day.[11,12] Cortisol should be lowest in the evening, allowing sleep at night. Due to the circadian nature of cortisol secretion, identification of cortisol dysregulation may not appear if measuring only total cortisol levels in blood at a single time point.

Cortisol and dehydroepiandrosterone (DHEA) are produced in closely related metabolic pathways. DHEA is an additional factor to consider in the development of type 2 diabetes. The production of both DHEA and cortisol is regulated by the release of adrenocorticotropic hormone (ACTH) from the adrenal cortex.[12] DHEA and DHEA-sulfate (S) are metabolic intermediates in the formation of the active sex steroids testosterone, dihydrotestosterone, and estrogen.[13] In human studies, exogenously administered glucocorticoids reduce basal and ACTH-stimulated blood levels of DHEA and DHEA-S.[14] Several studies have suggested that DHEA and DHEA-S are related to glucose and insulin regulation. A decrease of DHEA and DHEA-S is observed when humans are rendered hyperinsulinemic.[15,16] In addition, a reduction in serum insulin is associated with an increase in serum DHEA and DHEA-S.[17] Cortisol and DHEA can also be measured in saliva, and salivary levels have been found to correlate with plasma levels.[18] In clinical practice, some physicians order salivary cortisol and DHEA tests for patients who have or are at risk for diabetes, or to diagnose and monitor adrenal function.[19]

The salivary cortisol test requires patients to collect saliva samples at home four times during one day, in the morning, noon, afternoon, and at night. Things that may compromise the sample are discouraged, such as specific behaviors. For example, smoking, posture, and eating can all influence salivation and may thus introduce artifact into the sample.[20-24] As a result, patients are given very specific directions for collecting their saliva sample (described in detail in Methods section of this paper). A chart review was performed to examine the patterns of cortisol secretion and levels of DHEA in patients suspected of dysregulated glucose metabolism.

MATERIALS AND METHODS

Data was reviewed and collected from 29 patient charts from a naturopathic primary care clinic in Portland, OR. Informed consent for records review was obtained upon admission to the clinic. All data was coded to remove any identifiable information. In naturopathic clinics, individuals who are suspected of cortisol/DHEA dysregulation for reasons related to prediabetes or type 2 diabetes often undergo a clinical laboratory test called the Adrenal Stress Index™ (ASI™, Diagnos-Techs, Kent WA). ASI™ measures cortisol, DHEA, sIgA, and anti-gluten antibodies from saliva collected during the day. According to Diagnos-Techs, the analytic sensitivity

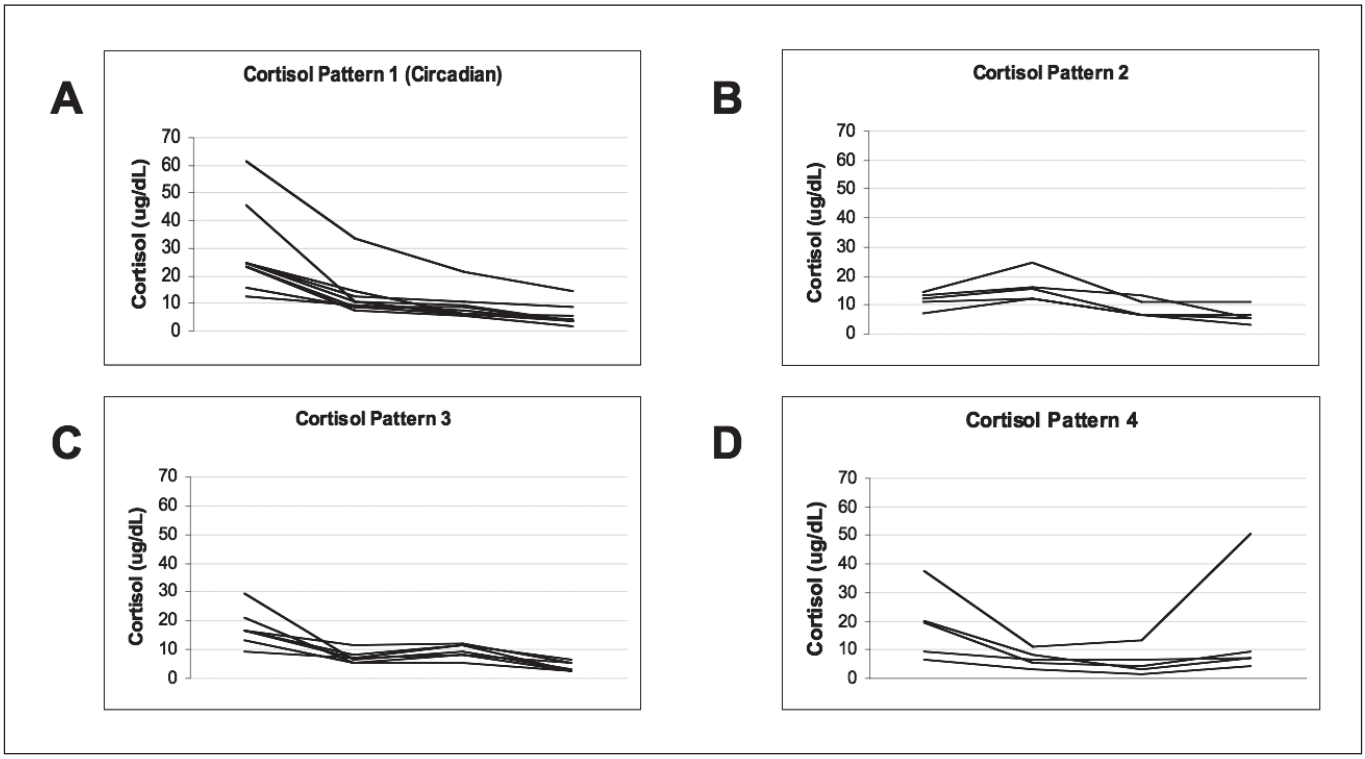

FIGURE 1

of this test is 0.8 nM to 1.0 nM and the specificity of the immunoassay to cortisol is at 99% or greater.[25] In diabetics and those at risk for diabetes, naturopathic physicians often do this test to help establish etiology of the disease. The chart review was done on patients who were predominantly untreated or uncontrolled diabetics, and patients presenting with symptoms that indicated insulin resistance and/or adrenal dysfunction whom had an ASI recorded in their chart. Additional patient care, number of visits, and information on medication intake was unobtainable for review.

Patients were instructed to follow the prementioned salivary collection protocol, but compliance was not assessed in the charts. Salivary samples were self-collected by the patient at four intervals in one day (between 7-8 AM, 11 AM-12 PM, 4-5 PM, and 11 PM-midnight). Patients were instructed not to eat or drink, use antacids, bismuth or mouthwash, or brush their teeth or smoke for 30-60 minutes before collecting the sample. They were also instructed not to eat more than one tablespoon of chocolate, onions, garlic, cabbage, cauliflower, or broccoli, or to drink coffee, tea, or caffeine on the day of collection. Patients were instructed to maintain a typical exercise regimen and activity level to obtain representative daily results. A sample consisted of saliva collected on a cotton roll held in the mouth until saturated and then placed in a 5 mL tube. Samples are refrigerated and mailed within 3 days. Samples are considered stable for a week at room temperature. The ASI™ tests were evaluated at Diagnos-Techs' lab in Kent, WA.

The saliva samples were analyzed for cortisol by ELISA. DHEA and DHEA(S) were analyzed by ELISA using pooled samples from the noon and afternoon time points. Data were entered and analyzed in Microsoft Excel. A total of 29 ASI™ tests in 29 patients were found. For 28 of these patients, DHEA levels were also available. Serum fasting blood glucose levels were available for 20 of these patients.

RESULTS

Cortisol Patterns

A normal circadian rhythm cortisol pattern is one in which there is a rise before waking (before 7-8 AM), and then a gradual decline throughout the rest of the day[26] (Figure 1A). Twelve patients had recognized circadian patterns of cortisol fluctuation, which will hereafter be referred to as Cortisol Pattern 1 (CP1). Of the twelve with CP1 whom were classified in the "normal" pattern, six had normal values at all four test time points, as well as normal total cortisol values or burden. Thus, of the 29 total charts reviewed, only 21% of the patients would be classified with normal cortisol values as well as normal patterns. The remaining six patients with a "normal" pattern of cortisol secretion (high waking and then decreasing over the rest of the day) had abnormal values at one or more time points.

Seventeen of the 29 total patients fell into our classification of dysregulated circadian cortisol patterns.

This consisted of patients with cortisol patterns that did not decrease in slope over the course of the day. Fourteen of these 17 patients had cortisol values out of normal range at one or more time points, or in total cortisol burden. The dysregulated patients' cortisol plots fell into three distinct patterns, which we will hereafter call Cortisol Pattern 2 (CP2) (Figure 1B), Cortisol Pattern 3 (CP3) (Figure 1C), and Cortisol Pattern 4 (CP4) (Figure 1D).

Patients grouped into CP1 begin with a burst of cortisol between 7 and 8 AM (between 13-23 nM), and drop off slowly throughout the day. Normal values are considered 4-8 nM between 11 AM and noon, 4-8 nM between 4 and 5 PM, and 1-3 nM between 11 PM and midnight.[27] It is notable that with the group of patients who presented with normal patterns, there was a large range in cortisol levels they excreted at any specific point. Seven patients in CP1 had elevated waking cortisol levels as shown in Figure 1A.

Those patients classified with CP2 tended to have abnormal circadian cortisol levels, higher in the late morning (11 AM to noon), compared to higher waking levels. Their levels then dropped off or stayed the same later in the day and at midnight. In contrast, patients grouped into CP3 had peaks between 7-8 AM and a more significant drop at noon. This created a second "mini" peak on the graph between 4-5 PM, though the levels are actually closer to normal (Figure 1B). Patients assigned with CP4 fell into a distinct pattern of increasing between 11 PM and midnight (Figure 1D). While a normal value falls between 1-3 nM for this time point, these patients averaged 15.4 nM (SD 8.3). One of the patients had extremely high cortisol levels at two time points, including time point 4 (between 11 PM and midnight). If the data is reanalyzed without this patient, the average cortisol value is still high with an average of 6.75 nM (SD 2.06).

Typically, the highest cortisol value of the day occurs at the waking time point. Yet, of the patient charts reviewed, if levels were low at all, they were low at this time point. One patient with low total cortisol had consistently low cortisol levels, except the late night point, which is typically the lowest cortisol level (Figure 1D). There were three patients who had consistently high cortisol levels (Figure 1A and 1D), and two of these also had high total cortisol values.

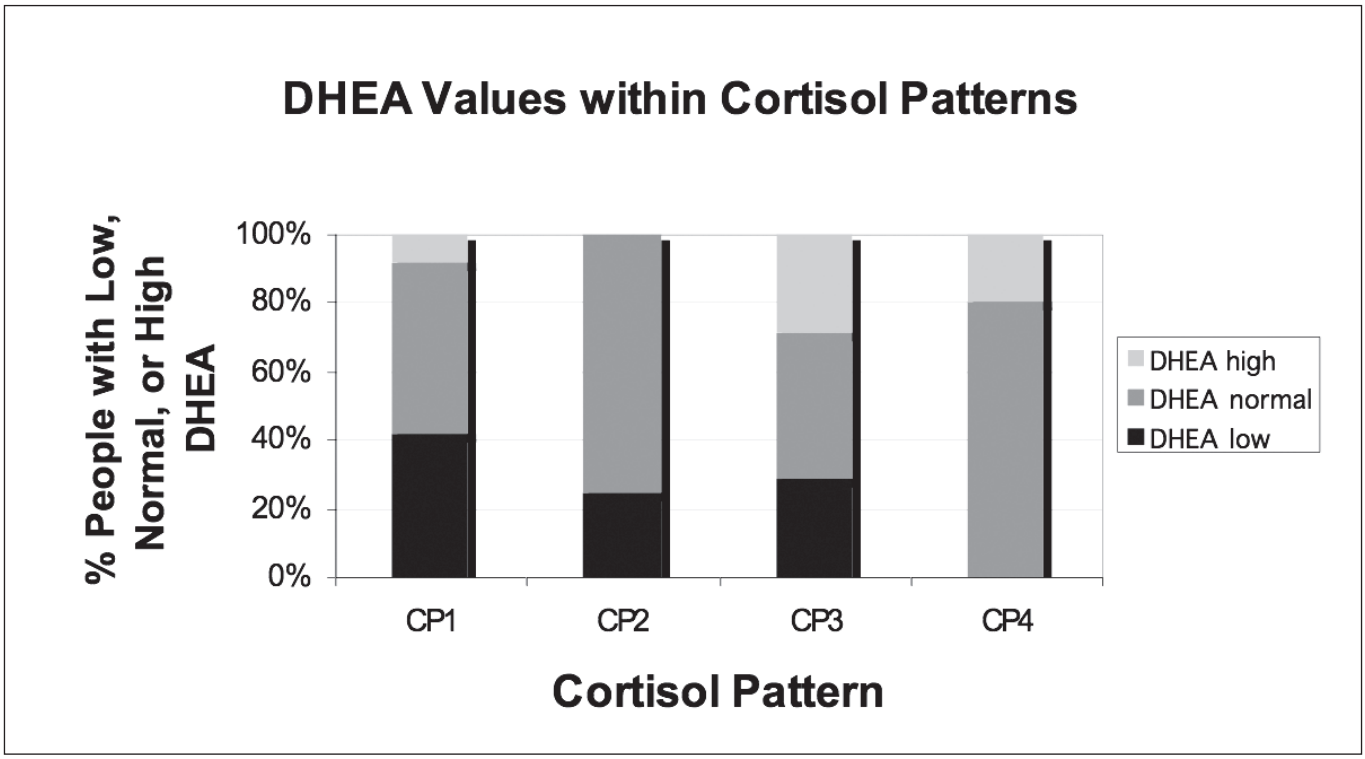

FIGURE 2 DHEA Values within Cortisol Patterns

DHEA and DHEA(S) Levels Compared to Cortisol Levels

DHEA levels were assessed in 28 of 29 patients. No consistent pattern emerged to correlate DHEA and cortisol (Figure 2). When comparing cortisol patterns to DHEA levels, patients who had normal cortisol patterns (CP1) did not necessarily have normal DHEA levels. Low, normal, or high DHEA and DHEA-S values were observed in the CP1 group. Similarly, low, normal or high DHEA levels were observed in the CP3 group. It is interesting to note that patients with CP2 have normal or low DHEA levels, but not high DHEA levels. In contrast, patients with CP4 have only normal or high DHEA levels. There were not enough patients in any group to observe statistical correlation between DHEA levels and cortisol patterns.

When comparing individual total cortisol levels with individual DHEA levels, four patients, who had high cortisol values at any particular time point, had normal DHEA values. Two patients, who had a high total DHEA value, also had high cortisol at all times during the day. One patient, who had a low DHEA value, had all normal cortisol values. Two patients, who had all high cortisol times throughout the day, also had a high total DHEA value.

Serum Fasting Blood Glucose

Twenty of the charts contained Serum Fasting Blood Glucose (SFBG) levels. Values were obtained on a separate date close to ASI™ collection. There were some variations seen in SFBG levels between those with an overall normal cortisol pattern and those with dysregulated patterns. However, as with DHEA, in these patients there was no correlation between SFBG levels and cortisol patterns (data not shown). Most patients in this chart review had normal SFBG levels. It is speculated that some may have been taking medications or nutraceuticals to manage glucose levels, but this data was not consistently charted or collected.

DISCUSSION

Cortisol

There is controversy about how and when cortisol should be measured.[28, 29] Many labs evaluate serum cortisol levels at a single time point. Other labs recommend the examination of salivary cortisol levels at multiple time points throughout the day. Our results suggest that cortisol measured at a single time point does not necessarily reflect the heterogeneity of cortisol patterns exhibited by a patient population.

The significance of cortisol dysregulation is related to the action of cortisol. Cortisol alters blood glucose by affecting glucose transporters in peripheral tissues such as fat and skeletal muscle.[30] Thus cortisol can contribute to elevated blood glucose levels due to inefficient uptake of glucose in the peripheral tissues. While the effects of cortisol on glucose suggest that it may be involved in the cause or progression of type 2 diabetes, there is little clinical evidence to support this theory. However, cortisol has been linked to obesity, specifically central obesity where the excess fat is carried on the midsection of the individual, which is a risk factor for type 2 diabetes.[5,11,31-36] Higher levels of cortisol can increase glucose production in the liver, inhibit glycogen synthesis, increase lipid accumulation, and decrease insulin secretion.[5, 34, 37, 38] This combination of events is a probable contributor to the development of type 2 diabetes.

Other studies indirectly suggest that cortisol is dysregulated in type 2 diabetics. Atiea and colleagues demonstrated that type 2 diabetics do not undergo the dawn phenomenon.[39] Measuring blood cortisol hourly between midnight and 9 AM, this study demonstrated that cortisol levels were significantly higher in newly diagnosed diabetics, especially between 3 and 4 AM. The authors suggest that this early cortisol peak contributes to the absence of a dawn phenomenon. This chart review did not include the early morning time point and did not discriminate between newly diagnosed diabetes, long-term diabetes, and prediabetes.

The results and conclusions of this initial chart review do not lend themselves to using cortisol patterns to predict risk of insulin resistance, prediabetes, or diabetes. Further evaluation of subjects, including a more detailed patient history with more stringent analysis of collection (supervision of subject) is required in order to assess the connection between specific cortisol patterns, risk prediction, and disease severity.

DHEA

DHEA and DHEA-S are measurable in blood and saliva. Although the DHEA levels are reported to change with total cortisol concentration, DHEA-S is essentially stable throughout the day and thus is not displayed as a pattern.[40,41]

Since DHEA-S is hydrolyzed into DHEA, measurement of DHEA is difficult. Labs often report DHEA and DHEA-S values together to compensate for this relationship. Normal DHEA levels fall between 3-10 mg/mL.[27] Seventy-five percent of the patients in this chart review who have high

DHEA levels also have high cortisol levels, suggesting a dysregulation in the adrenal pathway. Alternatively, patients with low DHEA levels tend to have normal total cortisol. While several animal studies demonstrate the significance of dysregulated DHEA to diabetes, very few human studies have shown a relationship. In animal studies, DHEA has been shown to reduce weight gain in obese mice without affecting food intake.[42] Other animal studies suggest that DHEA is antidiabetogenic.[43]

Human studies that attempt to relate DHEA to obesity and type 2 diabetes are complicated by age and gender differences among study subjects. Previous studies have shown that diabetic patients with high serum levels of insulin have lower serum levels of DHEA and DHEA-S.[44] A negative correlation between DHEA and hyperinsulinemia have been repeatedly demonstrated.[45-47] DHEA is thought to have lipomobilizing effects, targeting specifically the visceral fat. If this is the case, DHEA is an antagonizer of the effects of cortisol.[41]

METHODOLOGICAL ISSUES

In this chart review, there was a wide variance of values that made pattern analysis challenging. Primarily, sample collection and patient compliance were not assured despite strict saliva collection guidelines. The collection of salivary cortisol is complicated by other confounding variables that can affect the final values. It requires that patients adhere to other strict guidelines, as described in the methods section. The failure to follow these guidelines can lead to skewed or unrepresentative cortisol levels.

Patients completed their saliva collection at home and mailed the samples to Diagnos-Techs lab. The laboratory then measured cortisol levels and mailed the results back to the clinician. It was not possible to establish from a patient's chart whether the instructions were relayed to the patient or whether the patient followed instructions for optimal saliva collection. Thus, an abnormal value could mean that: 1) the patient's cortisol and/or DHEA is dysregulated, 2) the clinician did not properly instruct the patient, 3) the patient did not properly follow directions, or 4) there was a lab error.

Chart reviews pose an interesting challenge because patient compliance is not typically charted. Furthermore, chart reviews rely upon various clinicians to consistently collect similar data for their patients. Examination of the charts of 29 patients with obesity and diagnosed prediabetes or type 2 diabetes revealed a variety of missing data. Relevant to type 2 diabetes, some patient charts did not have a patient's weight, fasting blood glucose, or hemoglobin A1C.

CONCLUSIONS

We have demonstrated three distinct patterns of cortisol dysregulation suggesting that there are subsets of obesity, prediabetes, and/or diabetes patients to study more closely. The further identification of subsets of these patients should be done in a prospective observational study to avoid some of the methodological challenges and limitations inherent in a chart review. Future studies should also look at more standardized methods of salivary cortisol collection. The DHEA results suggest that some, but not all, cortisol patterns may correlate with specific ranges of DHEA and DHEA-S levels. However, a larger sample size must be used to verify this observation.

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