Exploring the Impact of Dark Chocolate Intake on Insulin Resistance, Stress Hormones, and Potassium Regulation

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The investigation into notable improvements in insulin resistance and stress hormones, with a specific focus on potassium levels, constitutes a captivating field of research. This study aims to delve into the potential influence of consuming dark chocolate on these critical physiological aspects. In aims, this research endeavors to comprehensively examine and assess the potential significant improvements in insulin resistance and the regulation of stress hormones and potassium associated with the intake of dark chocolate. In material and method, this study included 162 individuals aged between (40-60) years. They were divided into two groups. Group one contains 80 healthy people individuals without insulin resistance with a BMI less than 29kg/m2 and group two contains 82 patients with insulin resistance, with a BMI≥29 kg/m2. Twenty-five of them were given one bar of dark chocolate85% per day according to FDA, (Janssen Pharmaceutical Companies. (2019, January). for 45 days (Jansen EC,2023) both groups were analyzed serum glucose, insulin, serotonin, and cortisol before and after given supplement nutrients. Calories: Approximately 580 kcal per 100g, which provides the body with energy. Total Fat: About 43g per 100g, mainly from cocoa butter, which contains a mix of monounsaturated fats and saturated fats. Carbohydrates: Roughly 46g per 100g, acting as a primary energy source for our body. (Santos AC,2023)

In results, After conducting the statistical procedures for the results of the subjects for each of, HOMA-IR, cortisol, and serotonin it was found that there was a significant change with p-value (0.00), twenty-five donors resistance with insulin subjected daily one bar of 85% dark chocolate, resulting in significant improvements: decreased fasting glucose (84.12±5.6)mg/dl, insulin(12.74±4.38) µU/ml, HOMA-IR (2.57±1.02) cortisol(162.8±63.7 ) nmol/L, and increased serum serotonin levels (48.8±9.50) ng/ml, potassium (4.6 ± 0.20) (mg/dl). In conclusion, the statistical analysis, covering HOMA-IR, cortisol, and serotonin levels, revealed a significant change (p-value: 0.00). Daily consumption of 85% dark chocolate by twenty-five insulin-resistant donors resulted in substantial improvements, including reduced fasting glucose, insulin, and HOMA-IR. Cortisol levels decreased while serotonin and potassium levels increased respectively. and this achieves one of the sustainable development goals of the United Nations in Iraq which is (Good Health).

Keywords | insulin resistance, HOMA-1R, cortisol, serotonin, potassium, dark chocolate 85%.

Introduction: Exploring significant advancements in insulin resistance, stress hormone regulation, and the potential impact on potassium levels is a captivating journey within the domain of nutritional science (1). Dark chocolate, characterized by its abundant bioactive compounds, takes center stage as an intriguing subject for investigating its influence on these vital physiological elements. This study endeavors to unravel the complex interconnection between the consumption of dark chocolate and

the modulation of insulin resistance, stress hormones, and potassium regulation (2). Through a meticulous examination of these dynamics, the aim is to add valuable insights to the expanding understanding of the potential health advantages linked to integrating dark chocolate into dietary habits (3).

Materials and Methods:
This research is based on individuals with the age range of 40 to 60 years, initially enlisting participants exhibiting insulin resistance, and subsequently gathering data from control subjects devoid of insulin resistance. The participants were divided into two groups: Group One comprised 80 individuals without insulin resistance, serving as the control group, while Group Two consisted of 82 individuals with insulin resistance. A meticulous effort was made to match the gender distribution between the two groups. Baseline assessments of serum glucose, insulin, cortisol, potassium, and serotonin levels were conducted. Subsequently, 25 participants with insulin resistance were selected from the second group and were given one bar /day according to the FDA for 45 days. Post-supplementation, all measurements were repeated.

Result:
The lack of statistical significance (p > 0.05) underscores the careful alignment of the study's population in terms of age. Notably, the BMI ranges within the research participants reveal a significant distinction (p ≤ 0.05) between those without insulin resistance and those with insulin resistance. This observed difference implies a potential link between insulin resistance and obesity, in line with the main goal of the study. The intentional inclusion of individuals with insulin resistance, guided by the reference value of HOMA-IR, resulted in the expected outcomes during the comparative analysis of the two groups.

Table .1 Insulin resistance guide marker for individuals with and without insulin resistance expressed as mean±SD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Individuals without IR (N0=80)</th>
<th>Individuals with IR (N0=82)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.Cortisol (nmol/L)</td>
<td>212.2 ± 60.8</td>
<td>460 ±132</td>
<td>0.000[S]</td>
</tr>
<tr>
<td>S.Serotonin (ng/ml)</td>
<td>26.1±8.17</td>
<td>16.2±6.5</td>
<td>0.000[S]</td>
</tr>
</tbody>
</table>

S: Significant

Table .2 Comparative Analysis of Serum Cortisol and Serotonin Levels for Individuals with and without insulin resistance as mean ± SD.
S: Significant, n: number, S.F.G: Serum Fasting Glucose

There was a significant difference (p≤ 0.05) in the serum cortisol levels between individuals with and without insulin resistance, trending towards an increase with insulin resistance, although remaining within the normal reference range. Additionally, there were significant decreases (p≤ 0.05) in serum serotonin levels among those with insulin resistance.

Table (3) indicates that serum potassium levels are considerably lower (p≤0.05) in insulin-resistant individuals compared to healthy non-insulin-resistant individuals.

Table 3 Electrolyte analysis of Potassium (K) Levels in Individuals with and without Insulin Resistance (mean ± SD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy individual without IR (N= 80)</th>
<th>Individuals with IR (N= 80)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.K (mg/dl)</td>
<td>mean ± SD</td>
<td>mean ± SD</td>
<td>0.000 (s)</td>
</tr>
<tr>
<td></td>
<td>4.56 ± 0.59</td>
<td>3.25 ± 0.24</td>
<td></td>
</tr>
</tbody>
</table>

serum serotonin and potassium show a significant negative correlation(p≤0.05) with HOMA-IR while serum cortisol did not reach a significant level (p>0.5), but it can be noted, that it goes toward a positive direction.

Figure (1): The correlation between HOMA-IR and serum serotonin for individuals with insulin resistance.
In a research study, 25 individuals with insulin resistance were selected from the second group and administered a daily combination of one bar of 85% dark chocolate. This intervention led to noteworthy enhancements, including reduced fasting glucose, insulin, cortisol, and HOMA-IR, along with elevated serum serotonin, and potassium levels (p ≤ 0.05).

**Table 4** Insulin guide markers after 85% dark chocolate supplementation in 25 individuals with insulin resistance.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Individuals before supplementation</th>
<th>Individuals after supplementation</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No=25</td>
<td>No=25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>S.F.G (mg/dl)</td>
<td>103±11.5</td>
<td>83.12±5.60</td>
<td>0.000 (S)</td>
</tr>
<tr>
<td>S. Fasting Insulin</td>
<td>18.44 ± 7.6</td>
<td>12.75 ± 4.26</td>
<td>0.013 (S)</td>
</tr>
<tr>
<td>(µU/Ml)</td>
<td>421 ± 122</td>
<td>162.9 ± 63.70</td>
<td>0.00 (S)</td>
</tr>
<tr>
<td>S. Cortisol (nmol/L)</td>
<td>16.21 ± 6.6</td>
<td>48.9 ± 9.50</td>
<td>0.00000(S)</td>
</tr>
<tr>
<td>S. Serotonin (ng/ml)</td>
<td>4.76 ± 3.12</td>
<td>2.56 ± 1.02</td>
<td>0.005 (S)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>4.76 ± 3.12</td>
<td>2.56 ± 1.02</td>
<td>0.005 (S)</td>
</tr>
<tr>
<td>S.K(mg/dl)</td>
<td>3.0 ± 0.23</td>
<td>4.7 ± 0.20</td>
<td>0.000(S)</td>
</tr>
</tbody>
</table>

**Discussion:**
The influence of age was effectively addressed by meticulously selecting control group participants whose ages closely mirrored those of the patients. The non-significant results obtained provide support for the assertion that age not only correlates with insulin resistance and mitochondrial muscle dysfunction but is also associated with changes in body composition, potentially playing a role in the emergence of age-related insulin resistance. Obesity acts as a catalyst for diabetes linked to insulin resistance.
In individuals with obesity, the heightened release of non-esterified fatty acids, glycerol, hormones, and pro-inflammatory cytokines from adipose tissue may contribute to the initiation and progression of insulin resistance (4). The precise cause of insulin resistance remains unclear, but certain factors such as a family history of type 2 diabetes, excess weight—especially around the waist—and physical inactivity can heighten the risk. Elevated insulin levels, known as hyperinsulinemia, often occur as a consequence of insulin resistance. When the body fails to efficiently use insulin, the pancreas responds by increasing insulin production to counteract elevated blood sugar levels. Insulin resistance frequently serves as a common precursor to the onset of type 2 diabetes (5–7).

Within the realm of obesity, the balance of adipokines is disrupted, leading to increased levels of inflammatory adipokines that may play a role in the development of insulin resistance. Additional research suggests that the elevated circulating levels of free fatty acids, commonly found in obesity, have the potential to interfere with the insulin signaling pathway, thereby disrupting the typical cellular response to insulin (5).

Conversely, a sedentary lifestyle and unhealthy dietary habits play a significant role in the development of obesity and insulin resistance. Additionally, specific genetic variations have been identified as factors that can elevate susceptibility to these conditions (6).

Certain studies propose that cortisol plays a role in regulating glucose metabolism. It enhances the liver’s gluconeogenesis process, converting non-carbohydrate substances such as glycerol and amino acids into glucose. Elevated cortisol levels may result in increased blood glucose levels, as the liver releases more glucose into the bloodstream (7).

Another mechanism that depends on cortisol is linked to the distribution of body fat, particularly in chronic stress. Elevated cortisol levels are associated with an increase in central (abdominal) obesity (8). Central adiposity is recognized to be associated with insulin resistance and type 2 diabetes. Cortisol plays a role in influencing adipose tissue (fat) metabolism (9), leading to the breakdown of triglycerides into fatty acids released into the bloodstream. The excess presence of fatty acids in the bloodstream can disrupt insulin signaling, contributing to the development of insulin resistance (10).

This study refrains from endorsing either of the two proposed explanations for the potential association between low serotonin levels and insulin resistance. The first explanation suggests that low serotonin levels could result from insulin resistance, where compromised brain insulin, known for regulating dopaminergic pathways, may impact dopamine signaling. Consequently, this influence on the brain’s reward and motivation systems could potentially lead to symptoms associated with depression. Conversely, the second explanation attributes low serotonin levels to insulin resistance itself (11).

A deficiency in serotonin levels can result from either insufficient production by the body or ineffective utilization. Numerous factors, including genetics, stress, chronic pain, and nutritional deficiencies, are likely contributors to this imbalance (11). Additionally, research indicates a link between insulin resistance and elevated cortisol levels influencing dopamine. This connection is attributed to disrupted mitochondrial function, abnormal expression of monoamine oxidase, and increased dopamine turnover. Treatment with monoamine oxidase inhibitors has demonstrated the ability to reverse these effects. Consequently, brain insulin resistance is implicated in altering dopamine turnover, ultimately leading to the onset of anxiety and depressive-like behaviors (12).

Vigilant monitoring of potassium levels is essential for individuals with diabetes, given the crucial role of insulin in facilitating the transfer of potassium from the bloodstream into cells across
the body. Despite this inherent connection, the precise nature of the relationship between serum potassium concentration and insulin resistance remains insufficiently understood (13). Inadequate levels of potassium in the body may contribute to diminished insulin production, potentially resulting in elevated blood sugar levels. Studies suggest that individuals with low potassium levels tend to release less insulin, leading to higher blood sugar levels compared to those with normal potassium levels (14).

Through studies of low potassium levels, the finger may be pointed at obesity serum electrolytes can be influenced by obesity, leading to a higher occurrence of electrolyte imbalance in individuals with excess weight in contrast to the general population. Obesity contributes to the dysfunction of the Na+/K+-ATPase pump, with electrolyte imbalance being one of the outcomes of this impairment (15).

Conversely, dark chocolate is rich in flavonoids, particularly flavonols, known for various health benefits, including enhanced vascular function. While flavonoids have been proposed to have a positive impact on insulin sensitivity and glucose metabolism, the exact mechanisms behind these effects remain incompletely understood (16). An alternative perspective posits that the flavonoids present in dark chocolate could demonstrate vasodilatory effects, contributing to the dilation of blood vessels and promoting enhanced blood flow. This improved circulation may facilitate more efficient insulin delivery to tissues, subsequently augmenting insulin sensitivity. Studies suggest that dark chocolate might impact glucose metabolism by fostering the uptake of glucose by cells. Such a potential effect holds implications for the regulation of blood sugar levels and the reduction of insulin resistance, as indicated by certain research findings (17).

An additional proposition proposes that chocolate consumption could enhance insulin sensitivity, potentially prompting pancreatic beta cells to release more insulin. These dual actions hold the potential to lower blood sugar levels, as indicated by research findings, (18)

Dark chocolates with a high cocoa content contain serotonin. Consumption of dark chocolate can elevate blood levels of serotonin, contributing to improved mental well-being. Serotonin functions as an internal tension reliever and acts as an antidepressant (19). In simpler terms, chocolate, especially dark chocolate, has been demonstrated to facilitate serotonin production. Therefore, if you experience a sense of happiness after indulging in chocolate, it is not just a perception. Additionally, the cacao in chocolate is abundant in magnesium, promoting brain function and enhancing mood (20).

The consumption of chocolate triggers the release of four chemicals in the brain: oxytocin, serotonin, dopamine, and endorphins. Neurons use these substances to communicate with each other, potentially forming a connection with cortisol levels. The body tends to naturally produce more cortisol when there is an insufficient amount of dopamine in the system. This provides a plausible explanation for the reduction in cortisol levels. The impact of magnesium tartrate aligns with this process, flowing into the same stream (21).

The term "stress hormone" refers to cortisol, which is released in response to stress. Given the association between anxiety and heightened cortisol levels, magnesium has the potential to alleviate anxiety by lowering cortisol levels. Taurine also plays a pivotal role in regulating the neurotransmitter GABA (γ-Aminobutyric acid) (22). Providing a plausible explanation for the reduction in cortisol levels. GABA, crucial for inhibiting excessive neuronal activity, exerts an inhibitory influence on the stress response. Cortisol, released in response to stress, often shows elevated levels during stressful periods. By enhancing GABA activity, taurine may contribute to a reduction in the stress response, leading to decreased cortisol production (23). Following the
administration of magnesium tartrate and dark chocolate supplementation, a notable positive correlation was observed, approaching the accepted threshold of 0.057. To enhance statistical significance, it is advisable to broaden the study’s participant pool and undertake further research.

Insulin resistance can result from sugar addiction, where heightened insulin levels lead to the down-regulation of insulin receptors. These observations were noted following the administration of dark chocolate. It's worth highlighting that 90% of individuals with an anxiety disorder also experience depression, and 85% of those diagnosed with a major depressive disorder have comorbid anxiety disorders (24).

Conclusions:
In summary, the statistical analysis, covering HOMA-IR, cortisol, and serotonin levels, revealed a significant change (p-value: 0.00). Daily consumption of 85% dark chocolate by twenty-five insulin-resistant donors resulted in substantial improvements, including reduced fasting glucose, insulin, and HOMA-IR. Cortisol levels decreased while serotonin and potassium levels increased respectively. These findings collectively underscore the potential benefits of integrating dark chocolate into the daily diet of individuals with insulin resistance, demonstrating improvements in key physiological parameters.

Author’s declaration:
Conflicts of interest: None
We confirm that all tables and figures in this article are ours and written by the researchers themselves.
Ethical-Clearance: this manuscript approved by local ethical committee of physical education and sport sciences college for women on (February /2024)

Author’s contributions:
All contributions of this study were done by the researchers (R.H., M.J. and M.F.) who get the main idea and work on writing and concluding also with number of experts, Reem Hussein and Maysaa Jalal (College of Medicine, University of Baghdad) in Statistics, Huda Shihab in revision, Ibrahim Dabayebeh in proofreading

References:
استكشاف تأثير تناول الشوكولاتة الداكنة على مقاومة الأنسولين وهرمونات التوتر والبوتاسيوم

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جامعة بغداد – العراق


استكشاف تأثير تناول الشوكولاتة الداكنة على مقاومة الأنسولين وهرمونات التوتر والبوتاسيوم

إن التحقق في التحسينات الملحوظة في مقاومة الأنسولين وتنظيم هرمونات التوتر، مع التركيز بشكل خاص على مستويات البوتاسيوم، سيكون مجالًا بحثيًا جذابًا. تهدف هذه الدراسة إلى التعمق في التأثير المحتمل لاستهلاك الشوكولاتة الداكنة على هذه الجوانب الفسيولوجية الهامة. من حيث الأهداف، سيعود هذا البحث إلى إجراء فحص شاملاً وقناعي للتغييرات الكبيرة المحتملة في مقاومة الأنسولين وتنظيم هرمونات التوتر والبوتاسيوم المرتبطة بتناول الشوكولاتة الداكنة. ومن حيث المواد والطريقة، شملت هذه الدراسة 162 فردًا (أعمارهم بين 40-60 سنة). تم تقسيمهم إلى مجموعتين. تحتوي المجموعة الأولى على 80 شخصًا سليمًا (مؤشر كتلة الجسم أقل من 29 كجم/م2) بدون مقاومة الأنسولين. تحتوي المجموعة الثانية على 82 شخصًا مصابًا بمقاومة الأنسولين (مؤشر كتلة الجسم ≥ 29 كجم/م2). تم إعطاء الفردان في كل مجموعة قطعة واحدة من الشوكولاتة الداكنة بنسبة 85% في الأسبوع الأول. بعد ذلك، تم تحليل مستويات الجلوكوز في الدم، والأنسولين، والكورتيزول، والسيروتونين لكل من المجموعتين. وجدت هذه المراجعة أن هناك تغييرًا كبيرًا في مستوى HOMA-IR (2.57 ± 0.10) و HOMA-IR (2.57 ± 0.10) في المجموعة الأولى بعد التناول لـ 45 يومًا. في النتائج، بعد إجراءات التحليل، لم يتمكن أن نجد أي تغييرًا كبيرًا في مستوى الكورتيزول، والسيروتونين، والبوتاسيوم في المجموعة الأولى بعد التناول لـ 45 يومًا. مع ذلك، كانت هناك تغييرات طفيفة في مستويات الجلوكوز، والأنسولين، والكروماتين، والبوتاسيوم في المجموعة الأولى بعد التناول لـ 45 يومًا. هذه النتائج تدعم نتائج استطلاعات سابقة تشير إلى أن تناول الشوكولاتة الداكنة يمكن أن يحسن بعض الجوانب الفسيولوجية مثل مقاومة الأنسولين وتنظيم هرمونات التوتر والبوتاسيوم. هذا يحقق أحد أهداف التنمية المستدامة للإنسانية في العراق (الصحة الجيدة).