Dietary Stress and Energy Metabolism: Evaluation of the Adrenal Cortex

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**Competing Interests:**
There is no conflict of interest
Dietary Stress and Energy Metabolism: Evaluation of the Adrenal Cortex

Author(s): Shallie PD, Fakoya FA, Fakunle PB, Haruna MT, Shotunde DF

Abstract

The adrenal gland is an essential stress-responsive organ that is part of both the hypothalamic-pituitary-adrenal axis and the sympato-adrenomedullary system. The participation of the adrenal cortex in the control of carbohydrate metabolism is unquestionable, but the major pathways employed during deprived glucose states as in diabetes and starvation is not fully explained. A total of forty-eight healthy adult rats (Rattus Novergicus) of both sexes averages weight 200g were used. The rats were maintained on standard laboratory chow and water adlibitum. They were divided into four groups (n=12);

Group 1: Diabetic (D)
Group 2: Diabetic control (DC)
Group 3: Starving (S)
Group 4: starving control (SC).

Group 1: Diabetic were administered 70mg/kg body weight of aqueous solution of streptozotocin single dose intraperitoneally. After which they were given food and water liberally.

Group 2: Diabetic Control (DC), were administered equal volume of distilled water intraperitoneally and were given food and water liberally.

Group 3: Starving (S), were starved of food during the period of the experiment. The rats were sacrificed and the adrenal gland excised and processed for routine embedding and stained for H & E, while some portions of the gland were homogenized and used to determine LDH activities. The results showed significant increase in the activities of LDH in both the treated groups, hypertrophied cortex with hemorrhagic areas. In conclusion dietary stress induced by diabetes and starvation present with similar metabolic coping mechanisms.

Introduction

The adrenal gland is an essential stress-responsive organ that is part of both the hypothalamic-pituitary-adrenal axis and the sympato-adrenomedullary system. Chronic stress exposure commonly increases adrenal weight, but it is not known to what extent this growth is due to cellular hyperplasia or hypertrophy and whether it is sub region specific [1]. The existence of a functional relationship between the anterior pituitary, the pancreas and the adrenal cortex with respect to the control of carbohydrate metabolism is well established. The role of adrenal cortical hormones in the regulation of carbohydrate metabolism was pointed out by Britton and Silvette [2]. Long and Lukens [3] demonstrated the improvement in diabetes which results from adrenalectomy and Ingle [4] established the diabetogenic potency of pure cortical hormones. Although not all details of the mechanisms are known, participation of the adrenal cortex in the control of carbohydrate metabolism is unquestionable. [5]. Glucose production by gluconeogenesis is that main source of glucose during fasting and contributes significantly to hyperglycemia in diabetes mellitus [6]. Fasting and Diabetes are characterized by elevated glucocorticoids and reduces insulin respectively while stress responsiveness is impaired in diabetes [7]. Streptozotocin induced diabetes has been reported to cause an initial increase in the levels of Nitric Oxide Synthase (NOS) and NADPH-diaphorase in the adrenal gland of the rat [8]. Glucose – 6 – phosphate dehydrogenase levels have been reported to increase due to increased production of pentose sugars and NADPH needed for the synthesis of nucleotides [9 & 10]. While chronic food restriction is reported to result in moderate hyperadrenocorticism, which may play a role in activating cellular mechanisms that retard aging [11]. This study investigated the comparative effects of dietary stress (Diabetes and starvation) on adrenal energy metabolism as it relates to the metabolic pathways employed during these deprived glucose states.

Methods

Healthy adult rats (Rattus novergicus) of both sexes, average weight 200g were used. The rats were maintained on standard laboratory chow and water adlibitum for an initial three weeks period of acclimatization. Thereafter they were divided into four groups (n=12).

Group 1: Diabetic (D),
Group 2: Diabetic control (DC),
Group 3: Starving (S)
Group 4: Starving control (SC).

Group 1: Diabetic (D), was administered 70mg/kg body weight of aqueous solution of streptozotocin in a single dose intraperitoneally after which they were given food and water liberally.

Group 2: Diabetic control (DC), were administered equal volume of distilled water intraperitoneally and given water and food liberally.

Group 3: Starving (S) were starved of food during the period of the experiment, but were given water liberally.

Group 4: Starving control (SC), were given free access to food and water for the duration of the experiment.

The blood glucose level was monitored on days 1, 3, and 14 to establish the glycemic states in both groups, using GOD –POD method. Daily body weights were recorded so was food consumption. Four animals (n=4) from each group were sacrificed on days 3, 7 and 14. Some of the rats were sacrificed by cervical dislocation and the adrenal glands were quickly excised, weighed and appropriate portions made for quantitative estimation of LDH (Biolabo s. a. Fisms. France, code 92111). While others were sacrificed by an overdose of pentobarbital and the tissue processed for routine H & E [12]. The cells were estimated using the eyepiece micrometer procedure [13].

Results

Microanatomy: The adrenal cortex of the diabetic and starving group showed haemorrhagic spots, which are most marked at the cortico-medullary junction. (Plates 2 & 3). The adrenocortical cells showed significant increase in diameter (hypertrophy) on the experimental day 3(Table 1), while the increase in the subsequent days was not significant (Table 2). Table 3 presents the adrenal and the relative adrenal weights.

Biochemical: The activities of LDH in both treated groups showed steady significant increase from day 3 to 14 (Fig. 1) & (Fig. 2).

Discussion

The adrenal cortex of the treated groups showed hypertrophy and haemorrhagic spots (Plates 2 & 3). The haemorrhagic spots are most marked at the cortico-medullary junction. A large or moderate haemorrhage may be masked by other severe diseases especially in the neonates: sepsis with shock, CNS injury, asphyxia, intestinal obstruction and/or atresia, pulmonary hypertension, severe anaemia, or circulatory insufficiency, because their clinical manifestations are not specific only for adrenal injury damage. In severely damaged adrenal glands there is a dramatic onset of hypovolaemic shock, hypotension, abnormal thermoregulation, carbohydrate metabolism and water and mineral imbalance, tachycardia, arrhythmia, and cyanosis. Anorexia and rapid weight loss are present [14]. Hypertrophy was observed in which adrenal cholesterol; ascorbic acid concentrations, acid phosphatase and alkaline phosphatase activities were decreased, suggestive of active involvement of adrenal cortex in stress for homeostasis [15 & 16]. The dietary stress conditions such as starvation and diabetic conditions might cause a stress-like activation of adrenal cortex resulting in increased levels of glucocorticoids which in turn activate the intestinal Na+K+-ATPase activity [17]. Selve reported that the various internal organs, especially the endocrine glands and the nervous system, adjust to the constant changes which occur in and outside the body. This adjustment is referred to as the General Adaptation Syndrome. Selye concluded that the adrenals were the body's prime reactors to stress. He stated that the adrenals are the only organs that do not shrink under stress; they thrive and enlarge [18]. The increase in the activities of LDH noticed in the treated groups in this study (Figs 1 & 2) suggests the re-arrangements that the cell's metabolism undergoes in order to ensure survival. Recent studies suggested the metabolic strategy of stressed cell as a dynamic genetic and biochemical changes makes up a cell's response to a shortage of Coenzyme A (CoA), a key player in metabolism. [19]. Following the shutdown of CoA production, the cells quickly recycled CoA from other sources, extracting life-supporting energy from nutrients in the mitochondria. Low levels of CoA trigger the activation of genes that block other biochemical pathways that ordinarily use this molecule. Instead, the cell shifts most of the available CoA activity to producing glucose from the liver. Other organs then break down glucose into pyruvate inside mitochondria. In the mitochondria, CoA molecules feed pyruvate into a complex series of chemical reactions that produces molecules of ATP [19]. In conclusion dietary stress induced by diabetes and starvation present with similar metabolic coping mechanisms as demonstrated by the results of this study.

Reference(s)

1. Yvonne M. Ulrich-Lai, Helmer F. Figueiredo, Michelle M Ostrander, Dennis C. Choi, William C. Engeland, and James P. Herman. Chronic stress...
12. Drury RAB and Wallington EA. Carleton’s Histological Technique, 4th ... Histopathologic Technic and Practical Histochemistry, 1965
Illustrations

Illustration 1

Photomicrograph of Adrenal cortex showing radiating columnar cells of the zona fasciculata. Control group H&E x400
Illustration 2

Photomicrograph of Adrenal cortico-medullary junction showing haemorrhagic spots. Diabetic group H&E X400
Illustration 3

Photomicrograph of Adrenal cortico-medullary junction showing haemorrhagic spots. Starved group, H&E x400
Illustration 4

Diameter(um) of Adrenal cortical cells (zona fasciculata) after experimental day three (3)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (n)</th>
<th>Std Dev</th>
<th>Std Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diab. Cont.</td>
<td>4.17</td>
<td>0.65</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>0.030</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>5.10</td>
<td>0.26</td>
<td>0.11</td>
</tr>
<tr>
<td>Starv. Cont</td>
<td>4.17</td>
<td>0.65</td>
<td>0.26</td>
</tr>
<tr>
<td>Starvation</td>
<td>0.025</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>0.23</td>
<td>0.10</td>
</tr>
</tbody>
</table>

P is 0.05; Statistically Significant (SS)
Illustration 5

Diameter (um) of Adrenocortical cells (zona fasciculata) after experimental day seven (7)

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (n)</th>
<th>Std Dev</th>
<th>Std Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diab. Cont.</td>
<td>4.17</td>
<td>0.65</td>
<td>0.26</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starv. Cont.</td>
<td>4.38</td>
<td>0.68</td>
<td>0.28</td>
</tr>
<tr>
<td>Starvation</td>
<td>4.25</td>
<td>0.66</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.50</td>
<td>0.68</td>
<td>0.31</td>
</tr>
</tbody>
</table>

P is 0.05; not significant
Illustration 6

Effect of dietary stress on the relative Adrenal weight

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of treatment(days)</th>
<th>Body wt(g)</th>
<th>Adrenal wts(mg/pair)</th>
<th>Relative adrenal weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diab</td>
<td>3</td>
<td>175.00</td>
<td>0.027</td>
<td>0.31</td>
</tr>
<tr>
<td>Diab cont</td>
<td>3</td>
<td>115.00</td>
<td>0.070</td>
<td>0.61</td>
</tr>
<tr>
<td>Starvation</td>
<td>3</td>
<td>135.33</td>
<td>0.020</td>
<td>0.30</td>
</tr>
<tr>
<td>Starv cont</td>
<td>3</td>
<td>125.00</td>
<td>0.020</td>
<td>0.32</td>
</tr>
<tr>
<td>Diab</td>
<td>7</td>
<td>200.50</td>
<td>0.044</td>
<td>0.44</td>
</tr>
<tr>
<td>Diab cont</td>
<td>7</td>
<td>150.00</td>
<td>0.025</td>
<td>0.29</td>
</tr>
<tr>
<td>Starvation</td>
<td>7</td>
<td>185.33</td>
<td>0.070</td>
<td>0.38</td>
</tr>
<tr>
<td>Starv cont</td>
<td>7</td>
<td>120.00</td>
<td>0.032</td>
<td>0.27</td>
</tr>
<tr>
<td>Diab</td>
<td>14</td>
<td>20050</td>
<td>0.050</td>
<td>0.25</td>
</tr>
<tr>
<td>Diab cont</td>
<td>14</td>
<td>175.00</td>
<td>0.074</td>
<td>0.43</td>
</tr>
<tr>
<td>Starvation</td>
<td>14</td>
<td>115.75</td>
<td>0.080</td>
<td>0.69</td>
</tr>
<tr>
<td>Starv cont</td>
<td>14</td>
<td>185.00</td>
<td>0.070</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Diab=Diabetic; Diab cont=Diabetic control; Starv cont=Starvation control; wt=weight
Illustration 7

Activities of LDH in the Adrenal cortex of Starved group

The activities of LDH in the adrenal cortex of starved rats

<table>
<thead>
<tr>
<th>Period of treatment</th>
<th>Control</th>
<th>Starvation</th>
</tr>
</thead>
<tbody>
<tr>
<td>day 3</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>day 7</td>
<td>200</td>
<td>600</td>
</tr>
<tr>
<td>day 14</td>
<td>400</td>
<td>800</td>
</tr>
</tbody>
</table>
Reviews

Review 1

Review Title: Impact of induction of dietary stress by diabetes and starvation on adrenocortical anatomy and energy metabolism.

Posted by Faculty Dr. Pradip K Sarkar on 03 Jul 2012 02:34:12 AM GMT

What are the main claims of the paper and how important are they?:
The paper claims stimulation of dietary stress produced in streptozocin-induced diabetic condition and starvation alters anatomy of the adrenal cortex and its metabolic status.

This paper is interesting. The claim is not novel. It investigates the effect of dietary stress produced by induction of diabetes by streptozocin and starvation on adrenocortical microanatomy and lactic acid dehdrogenase activity. The fact that diabetes and starvation produce stress is well-known. Investigation on microanatomy of the adrenal cortex and LDH activity are interesting. However, the claims are not novel and the experiments were not designed neatly to address the question.

The claim is not properly placed in the context of the previous literature. The paper lacks presentation of previous work.

Results described support the claims, but not presented appropriately in a well-designed manner.

If a protocol is provided, for example for a randomized controlled trial, are there any important deviations from it? If so, have the authors explained adequately why the deviations occurred?
Protocol for experimental method is alright. Deviations and the results discussed are inadequate.

Methodology is valid. Only one comment I have the investigators used distilled water as a vehicle to inject to control and experimental rats. 0.9% saline water should be used to maintain physiologic osmolarity.

Experiments are inadequate to prove the claim. Additional well-designed experiments are needed.

Experiments are inadequate to prove the claim. Additional well-designed experiments are needed.

Rating: 3

Comment:
Methods: Experimental design is written poorly. It does not appropriately describe how long the animals were starved. It describes "...during the period of the experiment". It is not written clearly. If the starved rats were sacrificed on days 3, 7 and 14, no appropriate controls were described for this group clearly. Group 2 rats were injected with distilled water. Normally researchers use standard saline water (0.9% sodium chloride) or vehicle to treat corresponding control while doing i.p. injections. Also streptozocin solution is prepared in 0.9% saline to maintain a physiologic concentration. Using 0.9% saline would maintain physiologic osmolarity. Direct use of distilled water as vehicle is not recommended. GOD-POD method described in methods section has not been referenced in the text and is not described and this needs to be described. Methods section is mentioning recording of body weights and the food consumption. However the food consumption data has not been tabulated elsewhere in the manuscript. Although it is not required, showing and interpreting these data would have been interesting. Methods section describes weighing of adrenal glands and use of the appropriate portions of this tissue for LDH activity. It is important to describe which part of the tissue was used, how it was fractionated (tissue homogenate?) or which subcellular fraction was used and how it was prepared for biochemical assay of LDH activity. The purpose of measuring LDH is not discussed.

Results: The microanatomical photographs are not matching as described in the text as Plates 2 & 3. Instead they have been described as illustrations. A sequential and nice presentation of the data is a representation of a good scientific paper. This is not present in the current paper. Microphotographs are not very clear and they did not
clearly indicate the location of major focus of the data. Result from day 14 observation has not been described. Photomicrographs (microanatomy) of the adrenal cortex for the day 3-, 7-, and 14-day treatment have not been shown and would have been interesting. These data are relevant and important to the paper of interest. Result for LDH activity is described and this is labeled in illustration 7. It is not described as Figure 1 and Figure 2 as mentioned in the result section of the paper. Results on LDH activity for the diabetic and diabetic control groups have not been described. Data presentation with perfect and appropriate experimental design is very important to present scientific data.

Discussion: It lacks proper reference. Relationship among dietary stress, adrenocortical energy status and intestinal NaKATPase, their roles, and purpose of LDH assay are not discussed. This paper did not assay for coenzyme A, but discusses about it. Glycolysis breaks down glucose to produce pyruvate in the cytosol, not within the mitochondria. The authors mention: “CoA molecules feed pyruvate into a complex series of chemical reactions that produces molecules of ATP”. Pyruvate dehydrogenase converts pyruvate to acetyl-CoA that enters into the TCA cycle and electrons generated are passed through the electron transport chain and forms ATP. Overall, the discussion section is written poorly.

Competing interests: No

Invited by the author to make a review on this article? : No

Have you previously published on this or a similar topic?: No

Experience and credentials in the specific area of science:
20 years

How to cite: Sarkar P. Impact of induction of dietary stress by diabetes and starvation on adrenocortical anatomy and energy metabolism. [Review of the article 'Dietary Stress and Energy Metabolism: Evaluation of the Adrenal Cortex' by ]. WebmedCentral 1970;3(7):WMCRW002020
Review 2

Review Title: Dietary Stress and Energy Metabolism: Evaluation of the Adrenal Cortex

Posted by Dr. Olugbenga Ayannuga on 29 Jun 2012 02:23:28 PM GMT

What are the main claims of the paper and how important are they?:
The main claim is that the response of the adrenal gland to starvation and streptozotocin induced diabettes is similar from morphological and some biochemical point of view, the understanding of this assertion over a period of time might shed light on the morphological progression of streptozotocin induced diabettes pathogenesis as it affects the adrenal gland which is the major stress coping organ of the body.

The claims are novel and very illuminating especially in striking the similarity between starvation and streptozotocin induced diabettes with respect to the morphological changes in the adrenal gland.

The literature did not succinctly expose the obvious gap in knowledge, a further literature review will make the knowledge gap very conspicuous.

The result supports the claim of this work, however the presentation of the result will require an upgrade. For example the photomicrographs are not labelled, Illustration 1 did not indicate which of the controls (diabetic or starvation). The meaning of ME and ZR was not stated in illustration 2 and the hemmorhagic spots are not indicated. The photomicrographs are blurred generally, features are therefore very difficult to identify. Illustration 6 will need better arrangement, relative adrenal wt should be mean adrenal weight. Unit of measurement should be clearly defined e.g. um on illustration 4. The photomicrographs of the cortico-medullary junction of the 3, 7 and 14 day groups will tell the story better.

If a protocol is provided, for example for a randomized controlled trial, are there any important deviations from it? If so, have the authors explained adequately why the deviations occurred?
NA

The methodology is very valid, however the details are scanty, for example the starving regime was not explicit for the sake of reproducibility, the fixative used might make a whole difference and should therefore be stated. A strict demarcation between the sexes would have made for better discussion, sex hormones might have affected the outcome. The rationale behind the different method of sacrificing the animals should be explained.

Yes, the discussion will be more robust if the sexes are separated and a closer interval of sacrifice such as 1, 3, 5, 7, 9, 12 and 14 days is used. This might be difficult to add to this work but might form the focus of further studies in this direction.

Yes, the discussion will be more robust if the sexes are separated and a closer interval of sacrifice such as 1, 3, 5, 7, 9, 12 and 14 days is used. This might be difficult to add to this work but might form the focus of further studies in this direction.

Rating: 5

Comment:
This work is good, the author is encouraged to further the work along sex line as well as further the time line.

Competing interests: None

Invited by the author to make a review on this article?: Yes

Have you previously published on this or a similar topic?: No

Experience and credentials in the specific area of science:
NA
Review 3

Review Title: Dietary Stress and Energy Metabolism: Evaluation of Adrenal Cortex

Posted by Faculty Prof. Meral Guzey on 25 Jun 2012 09:39:28 AM GMT

What are the main claims of the paper and how important are they?:

Dietary stress, diabetes and its biochemical outcomes at Adrenal Cortex.

Format:
1. Abstract and the part of the methodology is repetitive. Abstract need to be re-written.
2. Results and Discussion can be re-written together.
3. There are format mistakes at reference list. Please be consistent with your reference writing format (please compare references 7 and 12).
4. Title needs to be specific.
5. Illustrations and the figures should be related to each other so the author can easily locate the figures and tables.

There are similar studies, but not the same methodological setting. This is the first study to show the direct relation between diabetes, starvation its relation to adrenal cortex, and LDH activity.

If not, please specify papers that weaken the claims to the originality of this one.

Similar work-example:
   Rebuffat P, Belloni AS, Malendowicz LK, Mazzocchi G, Meneghelli V, Nussdorfer GG.
   SourceDepartment of Anatomy, University of Padua, Italy.
2. Human Study: Diurnal behaviour of some salivary parameters in patients with diabetes mellitus (flow rate, pH, thiocianat, LDH activity)--note II.
   SourceCatedra de Fiziologie N. C. Paulescu, U.M.F. Carol Davila, Bucureti.

Yes

Yes

If a protocol is provided, for example for a randomized controlled trial, are there any important deviations from it? If so, have the authors explained adequately why the deviations occurred?

n/a

Yes

n/a

n/a

Rating: 4

Comment: n/a
Competing interests: No

Invited by the author to make a review on this article?: No

Have you previously published on this or a similar topic?: No

Experience and credentials in the specific area of science: none

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