Effect of Caloric Restriction on Metabolic Disorders and Pancreatic Apoptosis Associated with Type 2 Diabetes Mellitus in Adult Male Rats

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Abstract

Background: Insulin resistance (IR) and its concomitant relative insulin insufficiency is the cornerstone of type 2 diabetes mellitus (T2DM) but the exact pathogenesis that leads to IR is not completely clear.

T2DM is considered an explosive problem all over the world due to its dangerous complications which will further reflect passively on the socioeconomic conditions of the community.

As obesity is closely interrelated with IR, so, the effects of caloric restriction (CR) were documented to be beneficial on those having T2DM. Even, in some studies, CR had a significant improving effect on both serum glucose level and insulin sensitivity prior to its effect on fat distribution and changes in body weight.

Aim of the Work: Is to investigate whether caloric restriction can modulate some of the metabolic disturbances linked with T2DM or not and if it has any protective effect on pancreatic tissues.

Material and Methods: Thirty two male albino rats were divided into (GpI) control group, (GpII) type 2 diabetic (DM) group, (GpIII) (CR+DM) and (GpIV) (DM+CR) group. At the end of the experimental study we measured the BMI%, the serum glucose level, serum insulin level, HOMA-IR, serum triglyceride (TG) level and serum high density lipoprotein (HDL) level. Beside we evaluated the DNA ladder in the pancreas.

Results: Our results showed that CR either before or after induction of diabetes was associated with significant improvement of serum glucose, insulin, TG and HDL levels, and HOMA-IR. However, although BMI% was significantly decreased in GpIV yet it did not show any significant change in GpIII when compared to GpII.

Conclusion: 30% caloric restriction regimen led to significant improvement in T2DM and its related metabolic disorders and even can modulate its pathogenesis.

Key Words: T2DM – CR – TG – HDL – Pancreatic apoptosis.

Introduction

IR and extra-ordinary gluconeogenesis in the liver, are the crucial contributing factors to the manifestation of hyperglycemia in T2DM [1]. However, Miriam. [2], reported that in order to obtain a full-blown picture of T2DM, there must be pancreatic β-cell failure in addition to the prolonged IR.

Remarkably the prolonged exposure of the pancreatic β-cells to blood fats has a proinflammatory effect on the genes involved in insulin synthesis and may even totally damage pancreatic β-cell. So, the obesity is playing a key role in the pathogenesis of T2DM [3].

CR is a procedure used to counteract the deleterious effect of over nutrition, delaying or even preventing the occurrence of some metabolic related diseases such as T2DM, obesity and cardiovascular diseases [4].

The present work aimed to study the possible effect of 30% CR on T2DM associated metabolic disturbances and even on its pathogenesis.

Material and Methods

Thirty two male albino rats, approximately aged 8 weeks and weighed 150-200 gram were housed in wire mesh cages at room temperature under ordinary living conditions. Veterinary care was provided by laboratory animal house unit of faculty of Medicine, Cairo University. This work was performed in 2014.
Animals were randomly divided into the following 4 groups:

Group I: Control group (n=8): Rats were injected citrate buffer and no further life style modification was applied.

Group II: Diabetic (DM) group (n=8): Rats were fasted for 12-h before induction of diabetes. Streptozotocin (STZ) was freshly dissolved in 0.05 M citrate buffer, pH 4.5 and injected intraperitoneally in a single dose of 40mg/kg [8] with no further dietary modification for 1 month after induction of diabetes.

Group III: (30% CR+DM) group (n=8): Rats were subjected to 30% caloric restriction program for 1 month before induction of diabetes.

Group IV: (DM+30% CR) group (n=8): Diabetic rats were subjected to 30% caloric restriction program for 1 month after induction of diabetes.

At the end of the study protocol, all animals were weighed in grams and their naso-anus lengths in cm-while the rats were anesthetized with ether were measured to calculate their body mass index (BMI) as an index of obesity according to an equation formulated by Dubuis et al., [6]:

\[
\text{BMI} = \frac{\text{Cubic root of weight in grams}}{\text{Naso-Anal length in cms}} \times 1000
\]

Subsequently, fasting blood samples were withdrawn retro-orbital using a capillary tube for assessment of fasting serum glucose, fasting serum insulin, serum TG and serum HDL levels beside the calculation of (HOMA-IR). Afterwards, animals were sacrified followed by rapid excision of pancreas for further assessment of pancreatic DNA ladder (as an index of pancreatic apoptosis).

**Serum glucose level:** Was tested by kits supplied by “Diamond Diagnostics” [7]. The serum insulin concentrations: Were measured by enzyme immunoassay using the rat insulin ELISA kits [8] and HOMA-IR index: Was calculated as the product of fasting serum insulin (\(\muIU/L\)) and fasting serum glucose (mmol/L) divided by 22.5. HOMA-IR more than 4.0 is diagnostic of insulin resistance [9].

**The serum TG level:** Was measured by quantitative-enzymatic-colorimetric determination of triglycerides in serum [10]. However, HDL-Cholesterol: Is obtained through selective precipitation of LDL and VLDL lipoproteins, thus HDL lipoproteins remain in solution. HDL-Cholesterol in supernatant was treated as a sample for cholesterol assay, and after many reactions a colored compound was yielded. The color was measured at 546nm and was proportional to HDL-Cholesterol concentration in sample when used as directed [11].

**The DNA:** Was extracted from the pancreas using the kit supplied by Qiagen according to the manufacturer’s instructions. Then Gel electrophoresis was done for extracted DNA [12].

Data were coded and entered using the statistical package SPSS version 15. Data were summarized using mean, standard deviation for the quantitative variables. Comparisons between groups were done using analysis of variance (ANOVA) and multiple comparisons (Post Hoc test) for the quantitative variables, \(p\)-values less than 0.05 were considered as statistically significant [13].

### Results

**Comparison between BMI%, serum glucose (mmol/L), serum insulin (\(\muIU/L\)) levels and HOMA-IR in all studied groups:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gpl (Control)</th>
<th>GplI (DM)</th>
<th>GplII (CR+DM)</th>
<th>GplIV (DM+CR)</th>
</tr>
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<tr>
<td>BMI%</td>
<td>263.05 ± 9.68</td>
<td>280.06 ± 19.8</td>
<td>265.14 ± 8.62</td>
<td>255.61 ± 17.11</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.87 ± 0.18</td>
<td>12.81 ± 1.94</td>
<td>6.57* ± 2.21</td>
<td>6.51* ± 1.31</td>
</tr>
<tr>
<td>Insulin ((\muIU/L))</td>
<td>9.98 ± 1.24</td>
<td>17.88* ± 1.23</td>
<td>12.80* ± 1.20</td>
<td>10.74* ± 1.69</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.16 ± 0.24</td>
<td>10.34* ± 2.17</td>
<td>3.82* ± 1.62</td>
<td>3.138* ± 1.16</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD.

* Significant change comparing corresponding values to GplII.
* Significant change comparing corresponding values to GplI.

As observed in (Table 1): BMI% was insignificantly increased \((p=0.290)\) while fasting serum glucose (mmol/L), serum insulin (\(\muIU/L\)) and HOMA-IR were significantly increased \((p=0.000)\) in DM group (GplII) compared to control group (Gpl). Mean values in DM group were respectively 280.06±19.8; 12.81±1.94; 17.88±1.23 and 10.34±2.17 versus 263.05±9.68; 4.87±0.18; 9.98±1.24 and 2.16±0.24 in control group.

As shown in (Table 1) and Fig. (1), 30% CR for 1 month before inducing diabetes in GpIII had no significant effect \((p>0.05)\) on BMI% relative to both DM and control groups. Mean value was 265.14±8.62 in GplII; 280.06±19.8 in GplII and 263.05±9.68 in Gpl.
Values are represented as mean ± SD.

*: Statistically significant glucose level compared to corresponding value in control group (p<0.05).
#: Statistically significant insulin level compared to corresponding value in control group (p<0.05).
$*: Statistically significant HOMA-IR compared to corresponding value in control group (p<0.05).
@*: Statistically significant HOMA-IR compared to corresponding value in control group (p<0.05).
+*: Statistically significant TG level compared to corresponding value in control group (p<0.05).

Table 1 and Figs. (1,2) showed also that BMI%, fasting serum glucose level (mmol/L), fasting serum insulin level (IU/L) and HOMA-IR were significantly decreased (p<0.000) in GpIV compared to GpII. Mean values were respectively 255.61±17.11; 6.51±1.31; 10.74±1.69 and 3.18±1.16 versus 280.06±19.8; 12.81±1.94; 17.88±1.23; 10.34±2.17 in GpII.

Notably, no significant difference (p>0.05) was detected between these parameters in Gp IV and control group. Mean values were respectively 255.61±17.11; 6.51±1.31; 10.74±1.69; 3.18±1.16 in Gp IV versus 263.05±9.68; 4.87±0.18; 9.98±1.24; 2.16±0.24 in GpI.

Our study showed that there was no significant difference (p>0.05) between 30% CR for 1 month either before or after induction of diabetes as regarding serum glucose, serum insulin levels and HOMA-IR. Mean values were respectively 6.51±1.31, 10.74±1.69 and 3.18±1.16 in Gp IV; versus 6.57±2.21, 12.80±1.2 and 3.82±1.62 in Gp III.

Comparison between the serum TG (mg/dl) and HDL (mg/dl) levels in all studied groups:

<table>
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<tbody>
<tr>
<td>TG (mg/dl)</td>
<td>60.62±12.54</td>
<td>102.18±12.48</td>
<td>69.46±9.36</td>
<td>68.48±13.17</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>50.52±3.62</td>
<td>31.72±4.08</td>
<td>45.31±5.35</td>
<td>47.11±5.38</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD.
*: Significant change comparing corresponding values to GpI.
$: Significant change comparing corresponding values to GpII.

As shown in (Table 2) and Fig. (3), compared to control group, the serum TG level (mg/dl) was significantly increased (p=0.000), while the serum HDL level (mg/dl) was significantly decreased (p=0.000) in DM group. Mean values were respectively 102.18±12.48; 31.72±1.65 in GpII versus 60.62±12.54; 50.52±4.08 in GpI.

Comparable to its favorable effect on serum glucose, serum insulin, and HOMA-IR, application of 30% caloric restriction for 1 month before induction of diabetes lowered significantly (p=0.000) the serum TG and increased significantly (p=0.000) the HDL level relative to DM rats. Additionally, compared to GpI, a non-significant difference (p>0.05) was observed for both parameters. Mean values were respectively 69.46±9.36, 45.31±3.62 in GpIII versus 102.18±12.48; 31.72±1.65 in GpII and 60.62±12.54; 50.52±4.08 in GpI as shown in (Table 2).
Effect of Caloric Restriction on Metabolic Disorders

As revealed in Fig. (4) the DNA fragmentation pattern was monitored in treated and untreated pancreatic tissues on agarose gel electrophoresis. Necrotic strand breaks/streaking DNA was observed in DM group (GpII), but not in groups pretreated with CR (GpIII) prior to STZ exposure and also in diabetic rats treated with CR (GpIV).

Discussion

As T2DM has become a community threatening disease, so, novel therapeutic and even prophylactic strategies are in need [14]. Abnormal increase of gluconeogenesis in the liver is a main contributor to the hyperglycemia developed in patients with T2DM [15].

It is well recognized that CR can postpone aging and its related diseases including T2DM. So, CR mimetics may be considered as novel prophylactic and therapeutic agents in prediabetics or those having T2DM even those with complications [16].

Therefore, we induced diabetes in eight adult male rats (GpII) by single intra-peritoneal injection of 40mg/kg STZ, a well-known specific toxin causing pancreatic β-cell partial destruction [5]. It was in agreement with our results, in which there was marked pancreatic apoptosis obtained after induction of T2DM compared to control rats.

Consequently, the fasting serum glucose level was significantly elevated, indicating hyperglycemia compared to control group. Moreover, our results also showed a significant increase in fasting serum insulin level and HOMA-IR confirming the characteristic features of T2DM. Results may be explained by the incidence of IR in peripheral tissues such as skeletal muscles as they play a vital role in glucose homeostasis.

Our results were in agreement with Kadowaki et al., [17] who stated that in streptozotocin-induced T2DM, the tyrosine kinase activity of insulin receptor is severely reduced despite the up regulation in the number of insulin receptors.

Beside, obtained data for BMI% showed a non-significant increase in DM group compared to GpI. Our results could be primarily explained by the significant insulin resistance (hyperinsulinemia) which was in agreement with Takada et al., [18] who reported that diabetes associated polyphagia may be the cause of this non-significant weight change particularly that our rats in GpII were fed ad libitum and they might need a longer duration to reveal weight loss.

Changes in DNA ladder among all studied groups:

![DNA ladder among all studied groups](image)

Fig. (4): DNA fragmentation pattern in pancreas among all studied groups.
As over nutrition is strongly predisposing to certain chronic diet-associated diseases particularly T2DM and obesity so, Tuomilehto et al., [19] and Knowler et al., [20] have demonstrated the valuable effects of lifestyle modifications in avoiding the development of T2DM in prediabetics. They reported 5%-10% decline in the weight following dietary restriction. On the contrary, our results showed that only 70% caloric intake for 1 month before inducing diabetes (GpIII) had no significant effect on the weight relative to DM group. Results may be explained by the difference in the diet protocol used in our study. In contrast to previous reports who applied a low caloric diet with low fat content program, our work consisted of a non-specific CR diet. Thus, we suggest in further work to focus on the content of diet rather than the quantity in order to gain better results.

IR and T2DM are usually associated with multiple plasma lipid disorders including decreased HDL and increased TG levels [21]. Similarly, our results showed significant elevation of serum TG level and significant reduction of serum HDL level in DM rats versus the control group.

As well, Krauss., [22] emphasized that IR may play a critical role in the incidence of dyslipidemia associated with diabetes as insulin normally mediate the uptake of FFA by skeletal muscles. So, IR and the subsequent relative insulin lack will be associated with decreased FFA uptake via the skeletal muscles and their shift to the liver.

Beside, IR exerts a stimulatory effect on hepatic lipase enzyme which is authorized for breakdown of phospholipids into smaller LDL and HDL molecules and so diminishing the HDL level [22].

CR has been documented to realize significant improvement in both hyperglycemia and insulin sensitivity during weight reduction in obese T2DM patients and in preventing its occurrence in diabetes-liable rats [24]. In parallel, in this study, 30% CR over one month before induction of T2DM in GpIII realized a significant decrease in serum glucose level, serum insulin level and consequently HOMA-IR compared to GpII.

In agreement with our results, Pan et al., [25] who examined the impact of CR and exercise in 577 adults having IGT over a 6-years follow-up, they noted a significant reduction in the risk of diabetes (36% in the CR group versus 39% in the CR-plus-exercise group). Results of these studies were subsequently confirmed by the Diabetes Prevention Program (DPP) and the Finnish Diabetes Prevention Study [19]. They reported that both exercise and low calories diet improve insulin-mediated peripheral glucose utilization and thus lower the hyperglycemia. Accordingly, the improved insulin sensitivity will prevent further FFA accumulation and thus reducing their lipotoxic and proinflammatory effect on the pancreas which thereby delay P-cell damage, which was also documented in our study by the marked disappearance of pancreatic apoptosis in CR+DM group relative to DM group and even it is completely disappeared compared to control rats.

It is worth noting, although obesity and IR are correlated, but each of them, can individually predisposes to the development of lipid profile disorders [26]. Therefore, Pour And Dagogo-Jack., [27] suggested that CR is capable of inhibiting the development of T2DM and accompanying dyslipidemia. In accordance, our results revealed a significantly lower serum TG level (mg/dl) and a significantly higher HDL level (mg/dl) than diabetic rats (GpII) when exposed to 30% CR over a period of 1 month before induction of diabetes.

In support, the DPP Research Group., [28] assessed the impacts of CR on the precipitating factors of cardiovascular diseases in patients with IGT. Compared with the control group, CR group showed reduced blood pressure, improved HDL and TG levels. Additionally, Boardley et al., [29] revealed multiple improvements in lipid metabolism in those with IGT after 25% CR per day for 14 weeks, in the form of significant reduction in total cholesterol, LDL, TG, and non-HDL levels along with the decrease in apolipoprotein B concentration and proportion of apolipoprotein B/apolipoprotein A-1.

The beneficial effects of CR could be clarified by Angeliki and Leonard., [30] who noticed that CR activate certain intracellular signaling pathways which will further favor translocation of FFA transporting proteins helping for their further uptake and oxidation.

As we demonstrated the beneficial effects of prophylactic caloric restriction in T2DM rats, we also attempted to speculate the possible valuable curative effects of CR in T2DM rats. Our results revealed that BMI%, fasting glucose level, fasting serum insulin level and IR were significantly decreased after 30% caloric restriction for 1 month in diabetic rats (GpIV) compared to GpII.

Consistent with these findings, Bordone and Guarente., [31] found that early short-term period of CR produced significant decreases in fasting serum glucose and TG and raised both insulin
sensitivity and secretion in T2DM. However, following an extensive weight loss, they found that the effect of weight reduction was nearly equivalent to that gained with initial CR as regarding these parameters. Hence, they concluded that normalization of both pancreatic beta cell function and insulin sensitivity in T2DM was realized by CR alone independent of weight reduction.

As an extension to the valuable effect of CR in diabetic rats, the serum HDL level became significantly higher in (DM+CR) group relative to (GpII). Similarly, CR exerted an additional beneficial effect on serum TG level and lowered it significantly compared to corresponding value in GpII.

Similarly, Ugochukwu et al., [32] illustrated that CR was significantly effective in reducing body weight, serum glucose level, and TG concentrations in mild diabetic rats but without affecting the serum HDL level. However, the levels of HbAIC and HDL were non-significant in rats with severe diabetes. So, they concluded that CR is best effective in mild diabetic rats.

Furthermore, our results revealed that rats in (DM+CR) group showed complete disappearance of the pancreatic apoptosis compared with DM group.

In agreement with our results, Fernandez et al., [33] proposed that the effect of IGF-I on IGF-IRs in ß-cell of islets of pancreas with further stimulation of the IGF-I signaling pathway, would augment ß-cell proliferation in CR rats group. Moreover, gluco-kinase and insulin receptor substrate 2 may be involved in this process [34]. However, the expression of gluco-kinase and IRS-2 in the CR group compared with the control group, yielded non considerable differences. So, further studies are needed to inspect the definite effect of CR on ß-cells [35].

Lastly, which is more valuable: Prophylactic or curative caloric restriction in patients with type 2 diabetes mellitus? Unpredictably, our results showed no significant difference between the effects of the (CR either before or after induction of diabetes) regarding all the studied parameters.

Conclusions:

- Induction of diabetes was accompanied by adverse metabolic effects in the form of: Significant increase in the serum glucose level, serum insulin level and HOMA-IR with a concomitant dyslipidemia which was represented through the significant elevation of serum TG level, and significant decrease in serum HDL level and marked pancreatic apoptosis, while there was no significant change in BMI%.
- 30% CR regimen over 1 month prior to induction of diabetes was accompanied by significant improvement of all above distorted parameters in diabetic group, achieving near normal values except for serum insulin level in which there was still significant difference present relative to control group. Notably, there was no any significant change as regarding BMI%.
- 30% CR regimen over 1 month following induction of diabetes was associated with significant improvement of all above distorted parameters in diabetic group and moreover attained near normal values. In addition to the significant decrease in BMI% relative to diabetic group.
- No significant difference was demonstrated between the effects of CR either pre or post induction of diabetes.

Recommendations:

So, we recommend further studies for better understanding of the other mechanisms responsible for the beneficial effects of caloric restriction and to define the best caloric restriction regimen as regarding amount, composition and duration, and further research in other life style modifications which may offer potential solutions for pre-diabetics or diabetics even with complications.

References


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المختصر العربي

ان مقاومة الإنسولين التي تؤدي الى نقص الإنسولين نسبيا، هي حجر الزاوية في النوع الثاني من داء السكري ولكن الاحداث المرتبطة
الدقيقة التي تؤدي الى مقاومة الإنسولين هذه ليست واضحة تماما.

ويعتبر النوع الثاني من داء السكري مشكلة قائمة للانجاز على مستوى العالم بسبب المضاعفات الخطيرة المصاحبة له والتي سوف تتكمل
بصورة سلبية على الأحوال الاجتماعية والاقتصادية للمجتمع.

وكما هو معروف ان السمنة مرتبطة بشكل وثيق مع مقاومة الإنسولين. لذلك، فقد ثبت أن الحد من السعرات الحرارية سيكون مفيدا في
علاج النوع الثاني من داء السكري. فقد أثبت بعض الدراسات أن الحد من السعرات الحرارية قد حقق تحسن كبيرا في مستوى السكر في
الدم والحساسية للانسولين حتى قبل تأثيره على توزيع الدهون والتغيرات في وزن الجسم.

لذا فإن هذا العمل يهدف للتحقق من أن نظام الحد من السعرات الحرارية يمكن أن يقلل بعض الاضطراوات الإضافية المرتبطة بالنوع
الثاني من داء السكري أم لا. وقد تم اتباع 20% من ذكور المؤتيين الشابين، إلى أربع
مجموعات: المجموعة الأولى: الضابطة، المجموعة الثانية: تعديل اضطرابات الحالة، المجموعة الثالثة: التي تم تقليل السعرات الحرارية فيها بنسبة
20% عن المجموعة الضابطة لمدة شهر قبل اصابتها بمرض السكري، والمجموعة الرابعة، والتي تناول فيها الفئران المصابين فعليا بمرض السكري
من النوع الثاني، سعرات حرارية أقل من المجموعة الضابطة بنسبة 20% لمدة شهر.

في نهاية فترة الدراسة: قمنا بقياس مؤشر كتلة الجسم، مستوى الجلوكوز، الإنسولين، الدهون الثلاثية والبروتين الدهني على الكثافة
في مندول من يتناولون نظام حديد السكر. وربطنا النتائج مع انخفاض التأثير في البنكرياس واعترابه على درجة موت الخلايا المبرمج في البنكرياس.

وأظهرت النتائج أن برنامج الحد من السعرات الحرارية إما قبل أو بعد الإصابة بمرض السكري قد تؤثر على تحسين كبيرة في مستوى
الجلوكوز، الإنسولين، الدهون الثلاثية والبروتين الدهني على الكثافة في بنكنارس. ومع ذلك، وعلى الرغم من أن مؤشر كتلة الجسم انخفض بشكل كبير في المجموعة الرابعة لكن لم يظهر
أي تغير ذو نتائج إيجابي في المجموعة الثالثة، بالنسبة للمجموعة الثانية.

والخلاصة: هي أن تقليل السعرات الحرارية بنسبة 20% لampion نقص كبير في النوع الثاني من داء السكري واضطرابات التمثيل الغذائي
المصاحبة له بالإضافة لتأثير الإيجابي على خلايا البنكرياس.

الكلمات المفتاحية: النوع الثاني من داء السكري - الحد من السعرات الحرارية - الدهون الثلاثية - البروتين الدهني على الكثافة - موت
الخلايا المبرمج في البنكرياس.