ORIGINAL ARTICLE

Peripheral Insulin Resistance Induced by Streptozotocin and Modified Diets: Implications for Hippocampal Structural and Functional Integrity

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ABSTRACT

Background: An inverse association has been established between indices of insulin resistance and hippocampal structural and functional integrity.
Aim: We compared hippocampal-dependent function and morphology across rat models of insulin resistance induced by streptozotocin (STZ) with and without modified diets.
Methods: Rats were randomized to receive either multiple low-dose STZ (30 mg/kg; 5 successive days) with or without post-feeding with high-fructose drink (HFrD) or high-fat diet (HFD). At 30 or 60 days of such feeding, spatial memory was assessed by the Morris water maze technique, after which the animals were sacrificed. Fasting plasma insulin and glucose were then assayed, followed by estimation of homeostatic model assessment of insulin resistance (HOMA-IR). Moreover, the perfused brains of the rats were studied histologically by the Congo red technique. Oral glucose tolerance test was performed 48 hours to killing the rats by challenging rats with oral glucose (2 g/kg) followed by estimation of blood glucose at 0, 30, 60 and 90 minutes interval.
Results: The use of intraperitoneal STZ with or without modified diets triggered insulin resistance with variable degrees of biochemical, neurobehavioral and hippocampal structural perturbations that were most pronounced in the STZ-injected rats post-fed HFrD or HFD for 30 or 60 days; as opposed to those on STZ, HFD or HFrD alone.
Conclusion: These findings have implications and relevance for future studies aimed at exploring the association between insulin resistance and hippocampal structural and functional integrity.
Key words: Hippocampus, insulin resistance, high fat diet, fructose, streptozotocin

INTRODUCTION

Besides its peripheral effects in skeletal muscle, adipose tissue and liver, insulin also has central effects. Centrally, insulin receptor signalling promotes neuronal survival and memory formation by facilitating neurogenesis and synaptogenesis in the hippocampus[1]. Impairment of insulin activity in certain brain regions, specifically in the hippocampus, results in neurodegeneration[2,3], and does contribute to the pathogenesis of sporadic Alzheimer's disease[4-6].

In this regard, preclinical studies have demonstrated increased deposition of the toxic amyloid beta (Aβ) protein, hyperphosphorylation of tau, and formation of neurofibrillary tangle resulting from aberrant signalling of the insulin receptor via such mechanismsas impaired Akt/GSK3β activity[7-9]. Besides, Chang, Liang, Zhan, Lu, Shi, Qi, Feng, Wu, Sui, Zheng, Zhang, Sun, Bai, Li, Han[10] reported hippocampal insulin resistance and memory deficits resulting from the activation of endoplasmic reticulum stress with increased activity of the Jun NH_2-terminal

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stress with increased activity of the Jun NH_2-terminal kinases following the induction of peripheral insulin resistance and type 2 diabetes in rats injected with intraperitoneal streptozotocin and maintained on high-fat diet. Moreover, Agrawal, Zhuang, Cummings, Stanhope, Graham, Havel, Gomez-Pinilla [11] studied changes in insulin signalling in the hippocampal tissue of UCD-T2DM rats, and reported impaired hippocampal plasticity evidenced by significant reduction in BDNF-TrkB signalling. Related cognitive perturbation and hippocampal morphological aberrations have been demonstrated in different rodent models of peripheral insulin resistance and type 2 diabetes mellitus induced by diets or streptozotocin [5,12,13], and in post-mortem human brains [6,14].

To further decipher the cellular and molecular mechanisms underlying the association between insulin resistance (characteristic of metabolic syndrome and type 2 diabetes mellitus) and neurodegenerative disease (such as Alzheimer's dementia), future studies would include the use of animal models; where peripheral or central insulin resistance is induced by streptozotocin (STZ) or modified diets. Here, we employed cellular, behavioural and biochemical approaches to compare hippocampal morphology and functions across rodent models of peripheral insulin resistance induced by STZ injection with and without modified diets; with a view to assessing the severity of possible hippocampal dysfunction and dysmorphology.

MATERIALS AND METHODS

Chemicals

Animals:

Preparation of high-fat diet and high-fructose drink:

Induction of hyperglycaemia:

induced in rats after an over-night fast, using a low dose of intraperitoneal streptozotocin (30 mg/kg) (Sigma-Aldrich, St. Louis, USA) in chilled sodium citrate buffer (0.1 M, pH 7.4) for five successive days [17]. At 72 hours post-streptozotocin (STZ) injection, fasting blood glucose levels were measured by the glucose oxidase method using a glucometer (Accu-Check, Roche, Belgium). Animals with fasting blood glucose concentrations not less than 7 mmol/L were taken as hyperglycaemic and included in the study.

Administration of HFD and HFrD:

(n=6) were administered normal rat chow and non-sweetened water only. Two subsets of normoglycaemic rats (n=6 each) were fed HFD (Table 1) or given HFrD (15% fructose in water) *ad libitum* for 30 or 60 days. Moreover, two groups of STZ-induced hyperglycaemic rats were placed freely on either HFD (STZ+HFD; n=6) or HFrD (STZ+HFrD; n=6) for the same periods. The remaining hyperglycaemic rats were maintained on normal rat chow and non-sweetened water as streptozotocin (STZ) group.

Assessment of spatial memory and glucose tolerance:

After the behavioural test, rats were fasted overnight to assess glucose tolerance by the oral glucosetolerance test (OGTT). Fasted rats were challenged orally with glucose (2 g/kg body weight) [31], and blood was collected from the tail veins at 0, 30, 60, and 90 minutes post-glucose load to measure glucose concentrations using a glucometer (Accu-Chek, Roche, Belgium).

2.6. Fasting plasma insulin and glucose assays:

euthanasia (30 or 60 days of feeding with modified diets with and without STZ injection), rats were fasted over-night and anaesthetized with pentobarbital sodium[32]; thoracotomy was performed and blood was collected into heparinized tubes by cardiac puncture, centrifuged at 2500 x g for 10 minutes at 4°C, and the plasma was analyzed for fasting glucose and insulin. Fasting plasma insulin levels were quantified using rat insulin ELISA kit (Mercodia, Sweden), according to the manufacturer's instruction, with rat insulin as standard. Besides, fasting plasma glucose was measured by the glucose oxidase method using a commercially available kit (Span Diagnostic Chemicals, India).

2.7. Photomicroscopy for hippocampal histology:

anaesthetized rats were first subjected to whole-body perfusion with normal saline and then 4% phosphate-buffered paraformaldehyde solution (via cardiac puncture). The perfused brains were further fixed in the same fixative. The hippocampi were sectioned at 7 µm, and stained by the Congo red histological technique using a kit from Sigma (USA), according to manufacturer's instructions. Images were captured using the miniVID digital camera (LW Scientific, Lawrenceville, USA).

Homeostatic model assessment of insulin resistance:

plasma insulin and glucose, homeostatic model assessment of insulin resistance (HOMA-IR) was calculated as previously described [20], using the following formula (with glucose concentrations in molar unit):

HOMA-IR = (FPI x FPG)/22.5

Data analysis:

analysis of variance, followed by Bonferroni *post hoc* test, by means of the GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA). Results are presented as mean ± standard error of mean (mean

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± SEM). P-value of less than 0.05 ($p<0.05$) was taken as statistically significant.

RESULTS

Fasting plasma insulin and glucose:

Insulin sensitivity in the diet- or STZ-treated rats:

Spatial memory scores :

water maze experiments showed significant increases ($P<0.05$) in escape latency time in the STZ-injected rats that were maintained on high-fructose drink for 30 or 60days. Meanwhile memory scores in the STZ, HFD and HFrD groups did not differ significantly ($P>0.05$) from the controls (Fig. 4).

Hippocampal histology:

not demonstrate any Congophilic deposits in the hippocampus in all the groups at 60 days. In addition, no evidence of neuronal loss or hippocampal lesion was observed in all the groups except those administered high-fructose drink or high-fat diet with or without STZ injection (Fig. 4).

DISCUSSION

We have combined biochemical, neurobehavioural and histological techniques to compare hippocampal function and morphology across rat models of peripheral insulin resistance induced by intraperitoneal streptozotocin (STZ), with and without short-term feeding with modified diets. Post-feeding of STZ-injected rats with either high-fructose or high-fat diet produced notable insulin resistance as assessed by the homeostatic model assessment of insulin resistance (HOMA-IR) and oral glucose tolerance test (OGTT) at 30 or 60 days of feeding. These approaches, as previously characterized [21-23], offer a relatively quicker means of inducing peripheral insulin resistance in rats as opposed to the use of STZ or modified diets alone.

Streptozotocin is an islet beta-cell toxin that causes loss of beta cells via alkylation of DNA and generation of reactive oxygen species (ROS) [24], leading to chronic fasting hyperglycaemia. On the other hand, HFrD and HFD trigger hyperglycaemia and insulin resistance in peripheral tissues via mechanism that include increased release of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) [25], impairment of antioxidant defence system [26], increased formation of advanced glycation end products (AGEs) [27], and mitochondrial dysfunction [28,29], among others. Although the central and peripheral markers of ROS, inflammation and AGEs were not probed in the present study, a combination of beta-cell toxicity, coupled with elevated generation of ROS, AGEs and cytokines, may explain the much pronounced insulin resistance in the STZ+modified diet rats.

Furthermore, post-feeding of STZ-injected rats with modified diets for variable periods, in addition to being associated with significant insulin resistance, also produced noticeable impairment of hippocampus-mediated spatial memory at 30 or 60 days (as assessed by the Morris water maze tests) as opposed to those on STZ, high-fructose diet or high-fat diet alone. This implies that peripheral insulin resistance that is characteristic of type 2 diabetes mellitus, metabolic syndrome and obesity, has adverse effects on the central apparatus related to spatial memory, as previously characterized in other models [30,31]. Thus, in terms of the relative rapidity of impairing spatial cognition and learning in rats, a combination of STZ with modified diet has advantage over the use of STZ or modified diets only. In previous related studies, intracerebroventricular (icv) STZ triggered central insulin resistance that was associated with cognitive deficits and increased hippocampal tau phosphorylation mediated by decreased SIRT1 activity and accelerated phosphorylation of ERK1 and 2 in the hippocampus [32]. This important role of SIRT1 in diabetes-mediated cognitive defects was further corroborated by the recent findings of Agrawal, Zhuang, Cummings, Stanhope, Graham, Havel, Gomez-Pinilla [11].

Similar to the effects of STZ, diet-induced obesity is a known risk factor for metabolic syndrome and cognitive dysfunction in animal models [33]. In the present study, feeding of normoglycaemic Wistar rats with diets high in fat or fructose alone resulted in poor outcome in spatial memory test at 60 days; with marked impairment of this cognitive parameter in rats pre-injected with intraperitoneal STZ. Insulin resistance resulting from chronic feeding with HFD does produce cognitive dysfunction by mechanisms that range from dysregulated insulin signalling via the IRS1/PI3K pathway [34], to diminished antioxidant activity resulting from reduced NrF2 levels and activity [26], and failed activation of Akt and GSK3β resulting from serine phosphorylation of IRS1 at position 616 [33]. In this (HFD) model, Liu, Patil, Jiang, Sancheti, Walsh, Stiles, Yin, Cadenas [35] recently characterized the expression of the hippocampal GLUT3 and insulin-responsive GLUT4 glucose transporters in mice fed HFD for 12 weeks. Downregulation of GLUT3 and GLUT4 in these mice was associated with the suppression of the ERK/CREB pathway, with impaired long-term potentiation (LTP) in the cornu Ammonis 1 (CA1) region of the hippocampus. Moreover, elevated brain levels of inflammatory cytokines (IL-1β, IL6, and TNF-α) resulting from increased astrocytic and microglial activation, promote insulin resistance and learning

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impairment in rats fed high-fat/high-fructose diet[3], and in the diabetic and obese ob/ob mice [25]. This emphasizes the role of pro-inflammatory cytokines in the aetiopathogenesis of insulin resistance and its central complication.

Furthermore, in addition to the metabolic and hippocampus-mediated cognitive changes induced by insulin resistance, variable hippocampal structural modifications also occurred in the present study. At 60 days, insulin resistance induced by HFrD alone, STZ+HFrD or STZ+HFD resulted in some degrees of morphological alterations in the CA1 region of the hippocampus. Such untoward morphological changes are suggestive of impaired hippocampal dendritic and synaptic integrity in these rats, and lends credence to the markedly diminished cognitive performances in rats with insulin resistance induced by STZ+modified diets. Although immunohistochemical demonstration of synaptic and dendritic morphology could not be reported in our study, previous reports have established such changes. In rats fed a combination of high-fat/high-fructose/high-glucose diet to induce insulin resistance, Stranahan, Norman, Lee, Cutler, Telljohann, Egan, Mattson[36] reported that the diminished spatial memory scores in this model were associated with reduced BDNF in the hippocampus, with reduced spine density at the Schaffer collateral-CA1 synapses of the hippocampus. Moreover, Arnold, Lucki, Brookshire, Carlson, Browne, Kazi, Bang, Choi, Chen, McMullen, Kim [33] showed that feeding of rats with very HFD (60% kcal by fat) for 17 days or moderate HFD (45% kcal by fat) for 8 weeks, resulted in serine phosphorylation of IRS-1, with reduced expression of the postsynaptic scaffolding protein PSD-95 and synaptopodin in the hippocampus. This suggests reduced synaptic density resulting from diet-induced insulin resistance. Additional evidence in this respect came from the study by Calvo-Ochoa, Hernandez-Ortega, Ferrera, Morimoto, Arias[3] in rats fed HFD+HFrD. Such a diet triggered hippocampal insulin resistance that resulted in reduced synaptophysin expression and diminished synaptic spine density in the hippocampal CA1 region. Similarly, long-term feeding of rats with fructose-enriched diet resulted in poor expression of synapsin 1 and synaptophysin, with reduced hippocampal plasticity [37]. Besides, in adolescent and aging humans, there is a negative relationship between insulin resistance on the one hand, and hippocampal volume and brain structural integrity on the other[38,39]. Thus, such volume of evidence from human and animal studies bring to the fore the role of insulin resistance in the aetiopathogenesis of human cognitive dysfunction and neurodegenerative disease.

CONCLUSION

In the present rodent study, we have shown that peripheral insulin resistance is associated with biochemical, neurobehavioural and central morphological changes that are most pronounced in models induced by STZ with short-term feeding with modified diets, as opposed to those on STZ or modified diets alone. Such findings have implications for future studies directed at exploring the relationship between insulin resistance and hippocampal structural and functional integrity.

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Fig. 1: Fasting plasma glucose levels (A) and insulin concentrations (B) in the control (CTR), streptozotocin (STZ), or diet-treated rats at 30 or 60 days. *P<0.05 compared with control (CTR) group; ⁺P<0.05 compared with streptozotocin (STZ) group; #P<0.05 compared with high-fat diet (HFD) group; ^P<0.05 compared with high-fructose drink (HFrD) group.

Figure 1

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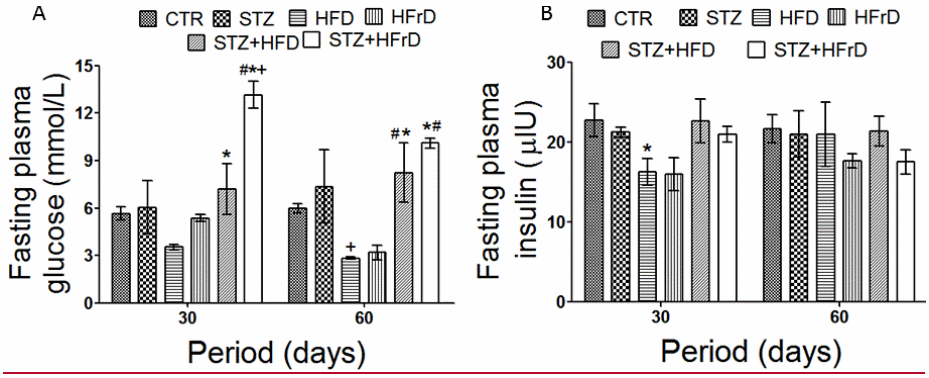

Fig. 2: Indices of insulin resistance and islet beta-cell function as assessed by the HOMA-IR (A) and HOMA-%β (B) methods in the control (CTR) rats and those treated with streptozotocin (STZ), high fat diet (HFD) or high fructose drink (HFrD) at 30 or 60 days. *P<0.05 compared with control (CTR) group; ⁺P<0.05 compared with streptozotocin (STZ) group; #P<0.05 compared with high-fat diet (HFD) group; ^P<0.05 compared with high-fructose drink (HFrD) group.

Figure 2

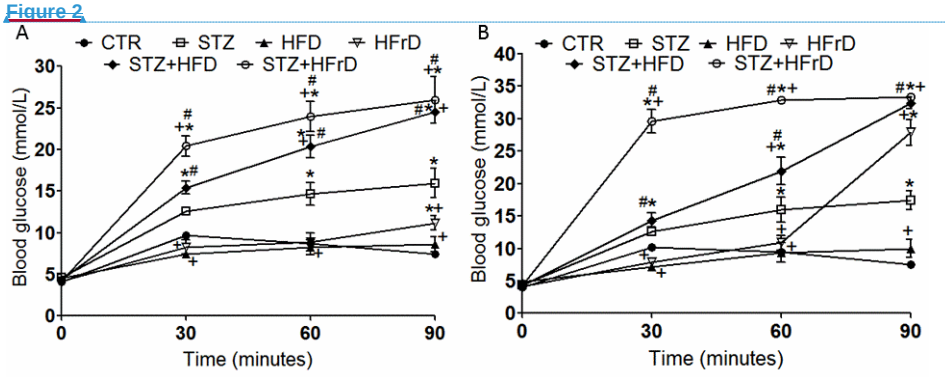

Fig. 3: Oral glucose tolerance test in the control (CTR) rats and those treated with streptozotocin (STZ), high fat diet (HFD) or high fructose drink (HFrD) at 30 days (A) or 60 days (B). *P<0.05 compared with control (CTR) group; ⁺P<0.05 compared with streptozotocin (STZ) group; #P<0.05 compared with high-fat diet (HFD) group; ^P<0.05 compared with high-fructose drink (HFrD) group.

Figure 3

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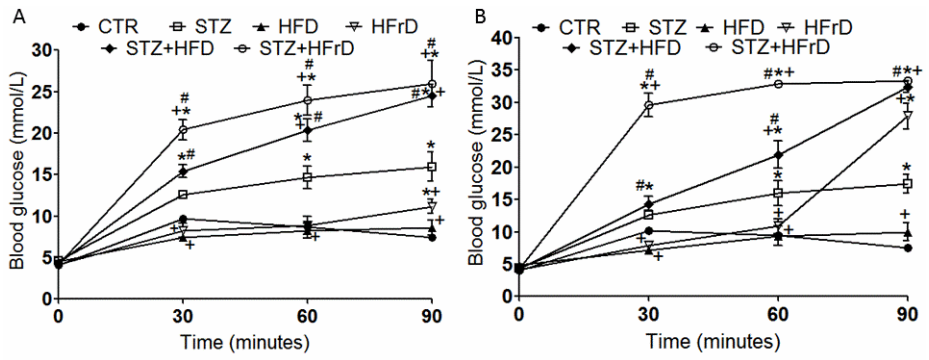

Fig. 4: Spatial memory scores in the control and fed rats, showing the worst memory scores in the STZ-injected rats fed high-fructose drink for variable periods (30d or 60d). *P<0.05 compared with control (CTR) group; ⁺P<0.05 compared with streptozotocin (STZ) group; #P<0.05 compared with high-fat diet (HFD) group; ^P<0.05 compared with high-fructose drink (HFrD) group.

Figure 4

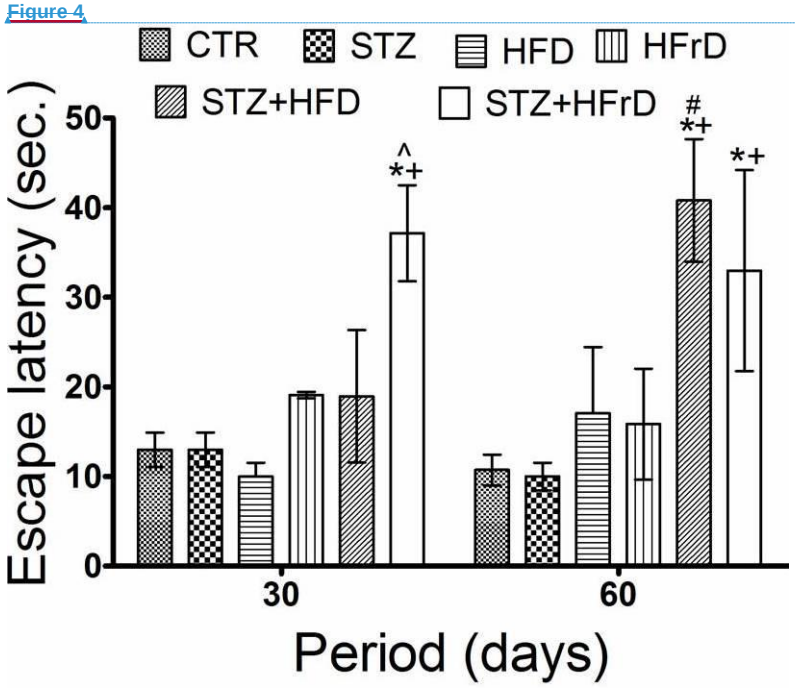

Fig. 5. Hippocampal CA1 region showing largely intact pyramidal neurons (arrow) in the control (A), STZ (B) and HFD groups at 60d. Pyramidal neurons in the CA3 region of the HFrD (D) and STZ+HFrD (F) groups showed poor cyto-architectonics and dysmorphology. Congo red stain, 400x.

Figure 5

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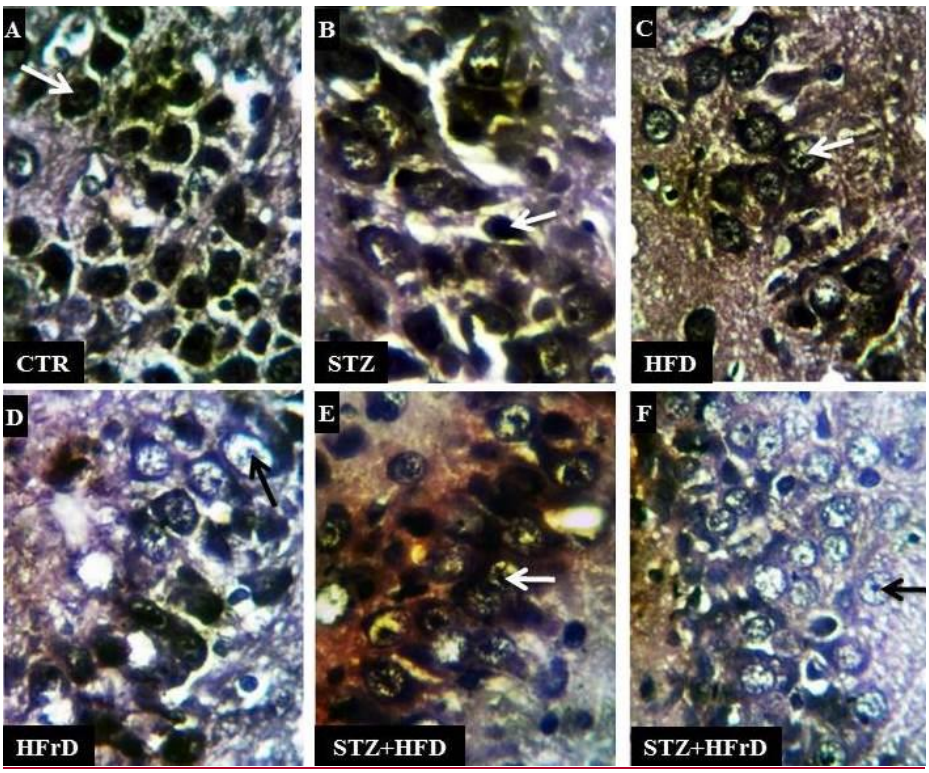

Composition	High Fat Diet (kg)	Normal Diet (kg)
Maize	5.5	5.5
Wheat offal	0.5	0.5
Groundnut cake	5.5	Nil
Soya meal	12.5 (toasted)	10
PKC	5	10
Bone meal	0.5	0.5
Fish meal	0.5	0.5
Methionine	0.025	0.025
Lysine	0.025	0.025
Industrial salt	0.0625	0.0625
Broiler premix	0.0625	0.0625

[Modified from Woods *et al.* (2003)]

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