Niger. J. Physiol. Sci. 32(June 2017) 47-54
www.njps.com.ng

Niger. J. Physiol. Sci.

Metformin Effects are Augmented by Chronic Intermittent Cold Stress in High Fat Diet Fed Male Wistar Rats

Ige A.O*., Iwaloye O.I., Adewoye E.O.
Applied and Environmental Physiology Unit, Department of Physiology, University of Ibadan. Ibadan Nigeria.

Summary: This study investigated the effects of metformin on some glucose regulatory indices in high fat diet (HFD) fed male Wistar rats exposed to room temperature and chronic intermittent cold stress (CICS). Thirty rats were randomly divided into 5 groups. Group 1(control) was maintained on standard rat chow while groups 2–5 were maintained on HFD for 8weeks respectively prior to experimental procedures. Control, group 2(HFD untreated) and group 3(HFD+metformin (250mg/kg) were exposed to room temperature while groups 4(HFD untreated+CICS) and 5(HFD+CICS+metformin) were exposed to CICS for 21days. Blood glucose was monitored before initial exposure to HFD and on days 1,7,14 and 21 respectively. Blood samples (5mls) were thereafter collected by cardiac puncture following light ether anaesthesia, serum was obtained and analysed for insulin, cortisol, and lipid profile using laboratory kits. Pancreatic β-cell function and insulin resistance were estimated using the Homeostasis Model Assessment equations. It was observed that blood glucose reduced significantly in groups 2-4 on day21 compared to day1 values. At day 21 post-treatment, insulin level and insulin resistance were increased while cholesterol levels were reduced in all HFD groups compared to control. Cortisol was increased in group 2 but reduced in groups 3-4 compared to control. HDL was reduced in groups 2-3 while liver glycogen was increased in groups 2, 3 and 5 compared to control. Beta cell function and muscle glycogen were increased while LDL and triglyceride were reduced in groups 2-4 compared to control. In conclusion, metformin ameliorates high-fat diet (HFD) induced impairment of glucose and lipid regulatory indices by facilitating an increase in the storage of glycogen in the liver and muscle. Chronic intermittent cold stress exposure in HFD rats does not ameliorate insulin resistance but reduces impaired glucose and lipid regulatory indices likely through an increase in adaptive thermo-genic mechanisms. The actions of metformin in reducing stressful stimulus and preventing pre-diabetes syndrome in HFD fed rats are augmented by exposure to chronic intermittent cold stress.

Keywords*:* Cold stress, Metformin, pancreatic beta cell function, high fat diet, glucose metabolism

**Address for correspondence*:* aby_ige@yahoo.com

Manuscript Accepted: June, 2017

INTRODUCTION

Exposure to cold in mammals elicits behavioural and physiological responses that minimize heat dissipation (e.g. vasoconstriction, huddling) and increase heat generation (e.g. shivering, activation of brown adipose tissue [BAT]) (Gordon, 2012). In addition to heat production during cold exposure, growing evidence suggests BAT may contribute to healthy metabolism in human (Lee et al., 2013). Studies have also shown that during cold exposure there is a tissue-specific regulation of the insulin-signalling pathway, which seems to favour heat-producing BAT and increase glucose uptake by muscle as well as white adipose tissue via an insulin independent pathway (Gasparetti et al., 2003). Cold exposure has also been reported to lead to increased food ingestion (Ohtani et al., 1999; Torsoni et al., 2003), lower blood leptin levels (Torsoni et al., 2003), higher blood catecholamine levels (Gabaldon et al 1995) and a transitory increase in blood thyroid-stimulating hormone (TSH), non-esterified fatty acids (NEFA) and corticosterone levels (Smith, 1984; Torsoni et al., 2003). It is therefore likely that increased food ingestion and lower blood leptin level during cold exposure may predispose to increased body weight, obesity and the metabolic syndrome.

Metformin, a frontline drug of choice in the management of diabetes mellitus, has been described as an effective drug to reduce weight in insulin sensitive and/or insulin resistant overweight as well as obese individuals (Bray and Greenway, 2007; Seifarth et al., 2013). It is known to act by decreasing the glucose output from the liver and increasing insulin sensitivity of body tissues. Whether this action of metformin is impaired or augmented by exposure to chronic intermittent cold stress is however unknown.

Increased urbanisation in most countries around the world has affected the quality and quantity of dietary intake leading to an increase in the consumption of high-fat and high-energy dense food substances (Swinburn et al., 2004). When combined with the increased exposure to chronic intermittent cold stress

(CICS) through daily exposure to air conditioning units in the working environment, this change in dietary conditions is often accompanied by reduced physical activities and a sedentary lifestyle, which may predispose people to obesity, the metabolic syndrome and diabetes mellitus (Swinburn et al., 2004). However, there is dearth of information on the exact effects of chronic intermittent cold stress and high-fat dietary consumption on glucose regulatory indices. Furthermore, whether the actions of metformin – a drug known to help control body weight and stabilize glucose level (Seifarth et al., 2013) – would be impaired or augmented in conditions of both high-fat dietary consumption and chronic intermittent cold stress is also yet to be investigated.

In this regard, this study investigated the effect of chronic intermittent cold stress on some glucose regulatory indices (body weight, blood glucose level, lipid profile, liver and muscle glycogen, cortisol, insulin, pancreatic beta cell function and insulin resistance) in Wistar rats. This study also assessed the likely hood of metformin in reversing the effects of high-fat diet alone and high-fat diet as well as CICS on the above listed glucose regulatory indices in Wistar rats.

MATERIALS AND METHODS

Experimental animals and grouping

Thirty (30) male Wistar rats (79 - 84g) were housed in standard, well aerated laboratory cages and maintained at 27±3° with 12-h light–dark cycles. They were initially fed for one week on standard rat chow from Ladokun Feeds, Nigeria (carbohydrate 67%, protein 21%, fat 3.5%, fibre 6%, calcium 0.8%, phosphorus 0.8%) and allowed free access to drinking water in accordance with the regulation and ethics regarding use of animals in the University of Ibadan. Thereafter, the animals were randomly divided into 5 groups of 6 animals each. Group 1 (control) was further fed on standard rat chow (Ladokun Feeds, Nigeria) while groups 2 -5 were fed on high-fat diet (HFD) (maize 19.1%, soya 19.1%, groundnut cake 19.1%, wheat 5.8%, fish meal 9.6%, calcium and phosphorus 1.9%, lysine 0.38%, methionine 0.38%, premix 0.76%, salt, 0.76%, lard 23%) and allowed free access to drinking water for 8weeks prior to experimental procedures.

Chronic intermittent cold stress (CICS) was induced using a modification of the method described by Dai *et al*., (2014) and Wang *et al.*, (2015). In brief, rats were transported in their home cages, with food, water and bedding, into a temperature-controlled chamber and exposed to 4°C for 1 hour (from 9 am to 10 am daily) and returned to the housing facility. The experiment lasted for 21 consecutive days. Group 1 (control) was fed on standard rat chow only and maintained at normal room temperature. Group 2 (HFD untreated) animals were maintained on HFD and exposed to normal room temperature. Animals in groups 1 and 2 were not given any treatment. Group 3 (HFD + metformin) animals were maintained on HFD, exposed to room temperature and orally treated daily with metformin (250mg/kg). Animals in group 4 (HFD + CICS) were maintained on HFD and exposed to CICS while animals in group 5 (HFD + CICS + metformin) were maintained on HFD, exposed to CICS and treated daily orally with metformin (250mg/kg). All animals were allowed free access to drinking water throughout the duration of the study.

Biochemical and Hormone analysis

Base-line blood samples were obtained in all groups before experimental procedures using the tail tipping method for glucose analysis. Blood samples were also obtained after high-fat dietary feeding for 8 weeks. This reading served as day 1 reading for the study. Thereafter blood glucose level was assessed on days 7, 14 and 21 respectively. The glucose level was assessed using an Accu-Check active glucometer (Tack et al., 2012) (Roche, Germany), based on the glucose oxidase method (Barham and Trinder, 1972). On day-21 post exposure to CIC, blood samples were obtained by cardiac puncture (after light di-ethyl ether anaesthesia) into plain sample tubes. The blood was allowed to stand at room temperature and thereafter centrifuged at 3000 rpm for ten minutes to isolate the serum. The serum samples were analysed for cortisol (BioVision, USA) and insulin concentration (CALBIOTECH, USA) using enzyme-linked immuno-absorbent assay (ELISA) kits. Serum cholesterol, high density lipoprotein (HDL), and triglycerides were determined by enzymatic procedures also using BIOLAB kit (France), while low density lipoprotein (LDL) was estimated using Friedewalds equation (Friedewald et al., 1972; Warnick et al., 1990).

Determination of liver and muscle glycogen

The liver and muscle glycogen content was determined by reacting fresh liver or muscle homogenate with anthrone reagent to form a blue-coloured solution that was read with a spectrophotometer at 630nm. The reading obtained was compared with that of known glycogen standards on a line graph to determine the actual glycogen concentration in each sample (Seifer et al., 1950; Jermyn, 1975).

Determination of insulin resistance and pancreatic beta cell function

The Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) and Pancreatic Beta Cell Function (HOMA-β) were determined mathematically using the following equations (Mathews et al., 1985):

$$\text{HOMA-IR} = \frac{\text{Insulin (µU/ml) x Glucose (mg/dl)}}{405}$$

$$\text{HOMA-}\beta = \frac{360 \text{ x Insulin (µU/ml)}}{Glucose\left(\frac{mg}{dl}\right) - 63}$$

Statistical Analysis

Data were analyzed using One-way ANOVA while Newman-Keuls' Post-hoc test was used to establish the statistical significance at $P<0.05$. Data were computed using Graph Pad Prism statistical software (5.04).

RESULTS

Body Weight Assessment in Control and Experimental Groups

Body weight (g) in group 1 (Control) was 171.1% increased after 8weeks of feeding on standard rat chow (Day 1) compared to their initial body weight (83.4±4.5 vs 226.6±8.3). Body weight values obtained in groups 2 (HFD untreated) (258.4±6.4), 3 (HFD + metformin) (262.8±6.1), 4 (HFD untreated + CICS) (237.0±13.4) and 5 (HFD + CICS + metformin) (263.2±6.2) were 225.8%, 213.6%, 164.63% and 220.2% increased respectively after 8 weeks of HFD feeding (Day 1) compared to their initial body weight (79.0±4.4, 83.8±3.0, 82.0±4.2 and 82.2±3.1) (Table 1). On day 21 after experimental procedures, animals in control group exhibited a 3.0% increase in body weight when compare to their corresponding day 1 values while body weight values obtained in groups 2, 3, and 4 exhibited a 4.2%, 4.0% and 5.6% increase in body weight respectively compared to their corresponding day 1 values. Body weight values obtained in group 5 showed a 7.1% reduction in body weight compared to their corresponding day 1 values (Table 1).

Assessment of Fasting Blood Glucose Level in Control and Experimental Groups

Blood glucose level (mg/dl) remained relatively constant in the control group throughout the duration of the study (78.5±3.2 vs. 74.0± 5.2). Significant increase in fasting blood glucose level was observed after 8 weeks of HFD feeding (Day 1) in groups 2 – 5 with values obtained being 21.5% (91.7±1.8 vs. 75.5±3.6), 13.1% (90.6±2.5 vs. 79.2±4.0), 48.9% (102.0±2.6 vs. 68.5±4.8) and 31.6% (94.5±5.1 vs. 71.8±3.7mg /dl) increased respectively compared to their initial fasting blood glucose values (Table 2). Within the groups, fasting blood glucose values obtained on day 21 after experimental procedures in groups 2,3 and 4 where 10.3%, 16.6% and 10.9% reduced respectively while values in group 5 were 6.7% increase compared to their corresponding day 1 values. Between the groups, blood glucose values in groups 2 (82.3±1.7), 4 (91.0± 4.0) and 5 (100.8±5.6) were increased while values in group 3 (75.6± 1.0) were comparable to controls (74.0± 5.2) on day 21(Table 2).

Table 1: Body weight changes in control and experimental groups

	Body weight (g)				
	Initial BW	**Day 1**	**Day 7**	**Day 14**	**Day 21**
Control	83.4 ± 4.5	226.6 ± 8.3	230.8 ± 8.7	233.8 ± 8.9	233.4 ± 8.4
HFD untreated	79.0 ± 4.4	258.4 ± 6.4*	256.8 ± 7.0*	254.6 ± 8.1*	269.2 ± 10.1*
HFD + Metformin	83.8 ± 3.0	262.8 ± 6.1*	262.8 ± 6.5*	262.2 ± 7.0*	273.4 ± 6.6*
HFD + CICS	82.0 ± 4.2	237.0 ± 13.4*	230.6 ± 13.8	242.0 ± 12.0	250.2 ± 12.6*
HFD + Metformin + CICS	82.2 ± 3.1	263.2 ± 6.2*	263.2 ± 4.4*	234.4 ± 5.6	244.4 ± 6.3

Values are mean±SEM. * indicates values that are significantly different from control values. Initial BW = initial body weight before initial exposure to either normal diet (group1) or high fat diet (group 2 – 5) for 8 weeks respectively. Control = Normal rats maintained on normal diet and temperature. HFD untreated = Untreated high-fat diet fed rats exposed to room temperatures. HFD + Metformin = Metformin treated high-fat diet fed rats exposed to room temperature. HFD + CICS= Untreated high-fat diet fed rats exposed to chronic intermittent cold stress (4^{o}C for 1hour daily for 21 days). HFD + Metformin + CICS = Metformin treated high-fat diet fed rats exposed to chronic intermittent cold stress (4^{o}C for 1hour daily for 21 days).

Table 2: Blood glucose level in control and experimental groups

	Blood glucose level (mg/dl)				
	Initial BG	**Day 1**	**Day 7**	**Day 14**	**Day 21**
Control	78.5 ± 3.2	74.4 ± 7.1	77.6 ± 4.3	81.6 ± 3.9	74.0 ± 5.2
HFD untreated	75.5 ± 3.6	91.7 ± 1.8	87.2 ± 1.7	81.3 ± 1.8	82.3 ± 1.7 ^{P}
HFD + Metformin	79.2 ± 4.0	90.6 ± 2.5	74.6 ± 3.4	81.8 ± 2.8	75.6 ± 1.0^{P}
HFD + CICS	68.5 ± 4.8	102.0 ± 2.6	69.4 ± 2.3	92.3 ± 5.1	91.0 ± 4.0*^{P}
HFD + Metformin + CICS	71.8 ± 3.7	94.5 ± 5.1	62.0 ± 3.9	88.8 ± 1.7	100.8 ± 5.6*

Values are mean±SEM. * indicates values that are significantly different from control values. ^{P} indicates values that are significantly different from their corresponding day 1 values within the group. Initial BG = initial fasting blood glucose level before initial exposure to either normal diet (group1) or high-fat diet (group 2 – 5) for 8 weeks respectively. Control = Normal rats maintained on normal diet and temperature. HFD untreated = Untreated high-fat diet fed rats exposed to room temperatures. HFD + Metformin = Metformin treated high-fat diet fed rats exposed to room temperature. HFD + CICS = Untreated high-fat diet fed rats exposed to chronic intermittent cold stress (4^{o}C for 1hour daily for 21 days). HFD + Metformin + CICS = Metformin treated high-fat diet fed rats exposed to chronic intermittent cold stress (4^{o}C for 1hour daily for 21 days).

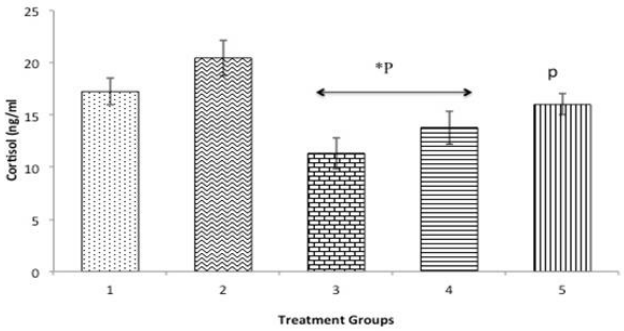


Fig. 1. Cortisol level in control and experimental groups. Values are mean ± SEM. * indicates values that are significantly different from control values. [P] indicates values that are significantly different from group 2 (HFD untreated group). Group 1 (Control) = Normal rats maintained on normal diet and temperature. Group 2 (HFD untreated) = High-fat diet untreated rats exposed to room temperatures. Group 3 (HFD + Metformin) = Metformin treated high-fat diet fed rats exposed to room temperature. Group 4 (HFD + CICS) = High fat diet untreated rats exposed to chronic intermittent cold temperatures (4ºC for 1hour daily for 21 days). Group 5 (HFD + Metformin + CICS) = Metformin treated high-fat diet fed rats exposed to chronic intermittent cold temperatures (4ºC for 1hour daily for 21 days).

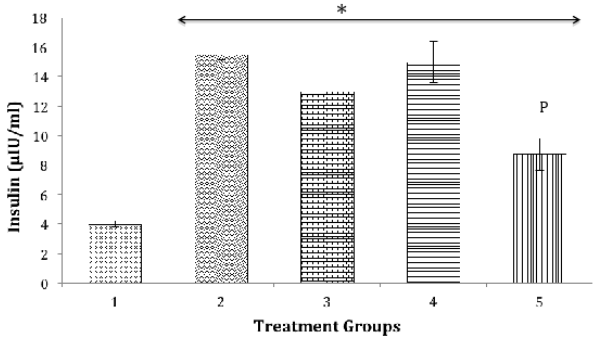
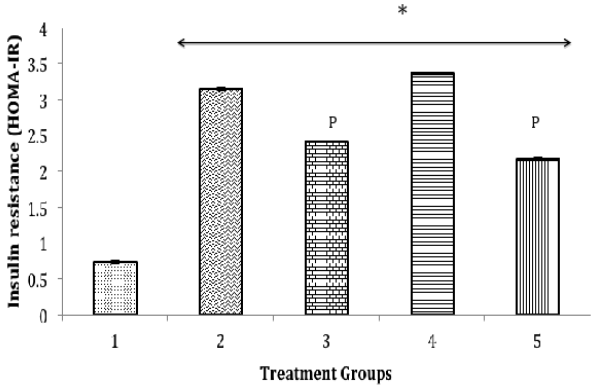


Fig. 2. Insulin level in control and experimental groups. * Indicates values that are significantly different from control values. [P] indicates values that are significantly different from group 2 (HFD untreated group). Group 1 (Control) = Normal rats maintained on normal diet and temperature. Group 2 (HFD untreated) = High-fat diet untreated rats exposed to room temperatures. Group 3 (HFD + Metformin) = Metformin treated high fat diet fed rats exposed to room temperature. Group 4 (HFD + CICS) = High-fat diet untreated rats exposed to chronic intermittent cold temperatures (4ºC for 1hour daily for 21 days). Group 5 (HFD + Metformin + CICS) = Metformin treated high-fat diet fed rats exposed to chronic intermittent cold temperatures (4ºC for 1hour daily for 21 days).



.

Fig. 3. Insulin resistance in control and experimental groups * Indicates values that are significantly different from control values. [P] indicates values that are significantly different from group 2 (HFD untreated group). Group (Control) = Normal rats maintained on normal diet and temperature. Group 2 (HFD untreated) = High-fat diet untreated rats exposed to room temperatures. Group 3 (HFD + Metformin) = Metformin treated high-fat diet fed rats exposed to room temperature. Group 4 (HFD + CICS) = High-fat diet untreated rats exposed to chronic intermittent cold temperatures (4ºC for 1hour daily for 21 days). Group 5 (HFD + Metformin + CICS) = Metformin treated high-fat diet fed rats exposed to chronic intermittent cold temperatures (4ºC for 1hour daily for 21 days)

Table 3: Liver and muscle glycogen level in control and experimental groups

	Liver glycogen (mg/100g fresh liver wt.)	Muscle glycogen (mg/100g fresh muscle wt.)
Control	136.2± 5.4	383.8 ± 20.5
HFD untreated	156.2 ± 5.4*	517.5 ± 14.8*
HFD + Metformin	196.4 ± 8.0*[P]	560.0 ± 19.8*
HFD + CICS	130.0 ± 5.4	545.8 ± 14.5*
HFD + Metformin + CICS	163.0 ± 5.5*	370.7 ± 15.6[P]

Values are mean±SEM. * indicates values that are significantly different from control values. [P] indicates values that are significantly different from group 2 (HFD untreated group). Control = Normal rats maintained on normal diet and temperature. HFD untreated = Untreated high-fat diet fed rats exposed to room temperatures. HFD + Metformin = Metformin treated high-fat diet fed rats exposed to room temperature. HFD + CICS= Untreated high-fat diet fed rats exposed to chronic intermittent cold stress (4ºC for 1hour daily for 21 days). HFD + Metformin + CICS = Metformin treated high-fat diet fed rats exposed to chronic intermittent cold stress (4ºC for 1hour daily for 21 days).

Assessment of Cortisol and Insulin Level in Control and Experimental Groups

Cortisol values (ng/ml) were significantly increased in group 2 (HFD untreated) (20.5±1.5), reduced in groups 3 (11.3±1.6) and 4 (HFD untreated + CICS) (13.8±1.0) compared to control (17.2±1.3). Cortisol values in group 5 (16.0±1.8) were comparable to control (17.2±1.3) (Fig 1). Insulin levels (µIU/ml) were increased in groups 2 (15.5±1.6, 3 (13.0±1.4), 4 (15.0±1.1) and 5 (8.7±1.2) compared to control (4.0±0.2) (Fig. 2). Insulin values obtained for group 5 were, however, significantly reduced compared to groups 2, 3 and 4 respectively (Fig. 2).

Pancreatic Beta cell function and Insulin Resistance in Control and Experimental Groups

Insulin resistance (HOMA-IR units) was increased in groups 2 (HFD untreated) (3.15±0.01), 3 (HFD + metformin) (2.42±0.01), 4 (HFD untreated + CICS) (3.37±0.02) and 5 (HFD + CICS + metformin) (2.17±0.03) respectively compared to control

Table 4: Lipid profile in control and experimental groups

	Lipid Profile			
	High density lipoprotein (mg/dl)	Low density lipoprotein (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)
Control	13.4 ± 2.4	64.3 ± 5.7	72.5 ± 3.9	77.7 ± 5.4
HFD untreated	6.9 ± 0.9*	48.5 ± 5.0*	54.7 ± 3.7*	55.3 ± 5.5*
HFD + Metformin	9.6 ± 1.3^{P}	42.6 ± 3.7*	54.5 ± 4.1*	52.2 ± 3.3*
HFD + CICS	11.0 ± 1.5 ^{P}	32.2 ± 7.3 ^{*P}	58.0 ± 3.2*	43.2 ± 6.1*
HFD + Metformin + CICS	12.5 ± 0.9	65.5 ± 6.0 ^{P}	62.3 ± 4.0	75.6 ± 6.9 ^{P}

Values are mean±SEM. * indicates values that are significantly different from control values. ^{P} indicates values that are significantly different from group 2 (HFD untreated). Control = Normal rats maintained on normal diet and temperature. HFD untreated = Untreated high fat diet fed rats exposed to room temperatures. HFD + Metformin = Metformin treated high-fat diet fed rats exposed to room temperature. HFD + CICS= Untreated high-fat diet fed rats exposed to chronic intermittent cold stress (4^{o}C for 1hour daily for 21 days). HFD + Metformin + CICS = Metformin treated high-fat diet fed rats exposed to chronic intermittent cold stress (4^{o}C for 1hour daily for 21 days).

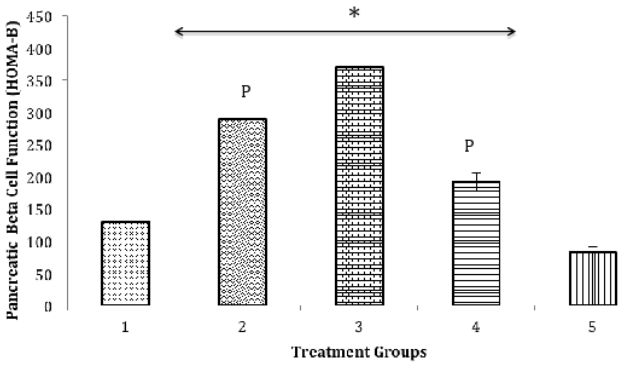


Fig. 4. Pancreatic beta cell function control and experimental groups * Indicates values that are significantly different from control values. ^{P} indicates values that are significantly different from group 2 (HFD untreated group). Group 1 (Control) = Normal rats maintained on normal diet and temperature. Group 2 (HFD untreated) = High-fat diet untreated rats exposed to room temperatures. Group 3 (HFD + Metformin) = Metformin treated high-fat diet fed rats exposed to room temperature. Group 4 (HFD + CICS) = High-fat diet untreated rats exposed to chronic intermittent cold temperatures (4^{o}C for 1hour daily for 21 days). Group 5 (HFD + Metformin + CICS) = Metformin treated high-fat diet fed rats exposed to chronic intermittent cold temperatures (4^{o}C for 1hour daily for 21 days).

(0.73±0.03). However, values obtained in group 3 and 5 were significantly reduced compared to group 2 and 4 respectively (Fig. 3). Pancreatic beta cell function (HOMA- β units) was increased in groups 2 (113.2%), 3 (183.2%) and 4 (47.4%) while group 5 values were reduced (36.4%) compared to control. Beta cell functions in group 4 and 5 were reduced compared to groups 2 and 3 respectively (Fig. 4).

Liver and Muscle glycogen content in Control and Experimental Groups

Liver glycogen content (mg/100g fresh liver weight) was significantly increased in groups 2 (HFD untreated) (156.2±5.4), 3 (HFD + metformin) (196.4±8.0) and 5 (HFD + CICS + metformin) (163.0±5.5) compared to control (136.2±5.4). Values obtained in group 4 (HFD+CICS) were comparable to controls (136.2±5.4) (Table 3). Muscle glycogen content (mg/100g fresh muscle weight) was significantly increased in groups 2 (517.5±14.8), 3 (560.0±19.8), and 4 (545.8±14.5) compared to control (383.8±20.5). Values obtained in group 5 (370.7±15.6) were comparable to controls (383.8±20.5) (Table 3).

Lipid profile in Control and Experimental Groups

Significant reduction in high density lipoproteins (HDL) was observed in groups 2 (HFD untreated) (6.9±0.9mg/dl) and 3 (HFD+metformin) 9.6±1.3mg/dl) while values obtained in groups 4 (HFD untreated + CICS) (11.0 ± 1.5mg/dl) and 5 (HFD + CICS + metformin) (12.5±0.9mg/dl) were comparable to control (13.4±2.4mg/dl) (Table 4). Significant reductions in low density lipoprotein (LDL) (mg/dl) and triglyceride (mg/dl) values were also observed in groups 2(48.5±5.0; 55.3±5.5), 3 (42.6±3.7; 52.2±3.3) and 4 (32.2±7.3; 43.2±6.1) while values in group 5 (65.5±6.0; 75.6±6.9) were comparable to control (64.3±5.7; 77.7±5.4). Cholesterol level in groups 2 (24.6%), 3 (24.8%), 4 (20%) and 5 (14.1%) were significantly reduced compared to control (62.3±4.0 mg/dl) (Table 4).

DISCUSSION

This study shows that feeding on high fat diet (HFD) in animals causes an increase in body weight, elevated blood glucose level, increased beta cell function, increased insulin secretion and increased insulin resistance which may predispose to the development of metabolic syndrome, obesity and type 2 diabetes if untreated. This is in accordance with the report of de Wilde *et al.*, (2009) who suggested that these observations in the HFD fed rats might be due to an increase in energy intake and a reduction in the metabolic rate of the animals. According to Ashcroft and Rorsman (2012), the effects of free fatty acid (FFA) arising from HFD on beta cell function are time dependent. Short-term exposure to FFA increases glucose stimulated insulin secretion (GSIS), which results in increased insulin secretion following a mixed

meal and enables storage of excess calories as fat, which contributes to overweight and obesity (Ashcroft and Rorsman, 2012). Free fatty acid has thus been reported to account for the compensatory increase of beta cell function in response to insulin resistance Stefanovski et al., 2011). It is therefore likely that the increase in insulin output and beta cell function in the HFD treatment groups may be attributed to overproduction of insulin from the pancreatic β cells in order to compensate for insulin resistance as has been reported to occur in the pre-diabetes state (Cerf, 2013; NIDDK, 2014). Furthermore, the observed elevated blood glucose level and hyperinsulinemia may also be attributed to increased HFD consumption which has been reported to cause nuclear exclusion and reduced expression of the transcription factors (Forkhead Box A2 (FOXA2) and hepatocyte nuclear factor 1 homeobox A (HNF1A) as well as a deficiency of GnT-4a glycosyltransferase expression in beta cells that results in hyperglycaemia, impaired glucose tolerance, hyperinsulinemia, hepatic steatosis and diminished insulin action in muscle and adipose tissues (Ohtsubo 2011). Interestingly, lipid profile observed in the HFD untreated rats in this study showed reductions in HDL, LDL, triglycerides and cholesterol at the end of the study despite an increase in body weight and blood glucose level, an observation that is at variance with other studies (Flatt et al., 1985; Jia et al., 2013) but is in accordance with the report of Zhukova *et al*., (2014) who reported increased levels of total cholesterol, triacylglycerols, LDL cholesterol, and very low density lipoprotein (VLDL) cholesterol in rats after 30 days of high-fat feeding which at 90 days showed a reduction in triacylglycerols and VLDL cholesterol level as well as elevated HDL levels. Lipid profile was assessed at the end of the experimental period and not intermittently; therefore, the exact effect of the hyperlipidemic diet on serum lipid profile may be considered obscured in this study. However, the values obtained at the end of the study show an increase in HDL levels in the metformin treated HFD, HFD cold stressed and metformin treated HFD-cold stressed groups respectively compared to control which in accordance with the report of Zhukova et al., (2014).

Exposure to cold has been observed to increase sympathetic tonus, resulting in reduced insulin secretion by pancreatic islets (Gasparetti et al., 2003) augmentation of BAT metabolic activity (Scarpace et al., 1996; Puigserver et al., 1998) and an increase in glucose uptake by white adipose tissue (Moreno-Aliaga et al., 2002). Exposure of HFD rats to chronic intermittent cold stress (CICS) at the end of this study reduced blood glucose level compared to day 1 but not control. It also reduced pancreatic beta function but did not ameliorate insulin resistance caused by high-fat diet feeding. Furthermore, liver glycogen level in cold stressed HFD untreated rats was not significantly different from control. It is likely that the in increased blood glucose level may be due to increased availability of energy substrates for increased muscular activity for thermogenesis arising from CICS. This suggests that CICS may optimize energy expenditure in high fat dietary conditions by improving glucose clearance rates through cold adaptation mechanisms in homoeothermic animals such as shivering, behavioural changes (cuddling and coiling up), increased brown adipose tissue (BAT) production, and increased metabolism and energy production. These factors have also been reported to be responsible for the improved glycaemic level in experimental rats exposed to cold stress (Gasparetti et al., 2003; Wang et al., 2015).

Metformin (1,1-dimethylbiguanide) is a biguanide derivative that is used to treat hyperglycemia in individuals with type-2-diabetes mellitus by acutely decreasing hepatic glucose production and increasing insulin action at specific tissues such as the muscle and fat (Moreno-Navarrete et al., 2011; Tahrani et al., 2011). It has also been described as an effective weight-loss drug in non-diabetic individuals with obesity (Seifarth et al., 2013). Treatment of HFD rats with metformin in this study did not reduce weight gain but lowered blood glucose level and increased liver as well as muscle glycogen content compared to control and HFD untreated. Furthermore, insulin level and insulin resistance was also reduced in the metformin-treated HFD group. These observations suggest that pre-diabetes or metabolic syndrome resulting from high-fat dietary ingestion could be ameliorated by oral metformin intake. Beta cell function in this study remained elevated in the metformin-treated HFD treatment group compared to HFD untreated group which is contrary to the reports of Pantane *et al.*, (2000) and Piro *et al.*, (2012) who reported that metformin is able to restore the intracellular abnormalities of glucose and free fatty acid (FFA) metabolism and to restore a normal secretory pattern in rat pancreatic islets whose secretory function has been impaired by chronic exposure to elevated FFA or glucose levels. In the metformin treated HFD cold stressed rats on the other hand had, reduced body weight, increased liver glycogen content but increased blood glucose level and reduced beta cell function compared to control was observed. Liver glycogen though increased in this group was not significantly different from HFD untreated. Muscle glycogen observed in this group was also insignificantly different from control but decreased compared to HFD untreated. It is likely that increased thermo-genic activity in response to cold stress such as shivering, behavioural changes (cuddling and coiling up), increased brown adipose tissue (BAT) production, and increased metabolism and energy production in this group could account for the observations in this group. This again suggests that

CICS augments metformin's action in ameliorating pre-diabetes and obesity resulting from increased HFD intake.

Cortisol is an adrenocortical hormone released in response to stress and low blood glucose concentrations. It increases blood sugar through gluconeogenesis, suppresses the immune system and aids in the metabolism of fat, protein, and carbohydrates (Hoen and Marieh, 2010). Cortisol level in the HFD untreated rats was increased suggesting the presence of stress stimulus in this treatment group which may be due to activation of low-grade chronic inflammation resulting partially from activation of the innate immune system and partly from an increase in blood glucose level that on its own triggers various inflammatory processes in the body (Ige and Adewoye, 2016). Cortisol levels observed in the HFD rats exposed to CICS were reduced compared to HFD rats that were kept at room temperature which is in accordance with the report of Wittert et al., (1992). Metformin treatment in HFD and cold stressed HFD groups also showed a reduction in cortisol level, which is in accordance with the report of Cho *et al.*, (2015) who reported that metformin may also exert its anti-hyperglycemic effect by activating AMP-activated protein kinase (AMPK) which then caused a decrease in adrenocorticotropic hormone (ACTH) and cortisol. These observations suggest that metformin alone or in combination with CICS reduced the stressor effects of high fat dietary intake in the body.

In conclusion, this study shows that HFD feeding induces changes in lipid and glucose regulatory indices resulting in the activation of stress-mechanisms in the body, increased body weight, pre-diabetes and metabolic syndrome. Treatment with metformin alone in high fat diet fed rats may improve blood glucose and lipid profile by either facilitating an increase in glucose uptake for storage as glycogen by the liver and muscle or prevent the breakdown of liver and glycogen stores in the liver and muscle. The improved glucose and lipid regulatory indices may then account for the observed reduction in stress level in the metformin treated HFD-fed rats. Under condition of chronic intermittent cold stress (CICS), HFD fed rats had improved lipid and glucose regulatory indices, which may be due to an increase in thermo-genic adaptations in HFD fed rats. In metformin treated HFD-fed rat exposed to CICS, the glucose and lipid regulatory effects of metformin were augmented by CICS with the exception of a reduction in muscle glycogen stores instead of an increase. Taken together, this study shows that the actions of metformin in reducing stressful stimulus and preventing pre-diabetes, increased body weight and metabolic syndrome in HFD fed rats maybe augmented by exposure to chronic intermittent cold stress.

REFERENCES

Ashcroft F.M., Rorsman P. (2012). Diabetes mellitus and the beta cell: the last ten years. Cell 148, 1160–1171.

Barham D., Trinder P. (1972). An improved color reagent for the determination of blood glucose by the Oxidase system. Analyst 97:142.

Bray G.A., Greenway F.L. (2007). Pharmacological treatment of the overweight patient. Pharmacol Rev; 59:151–184

Cerf M.E. (2013). Beta cell dysfunction and insulin resistance. Front Endocrine (Lausanne); 4 (37): 1 - 12 Doi: 10.3389/fendo.2013.00037

Cho K., Chung J.Y., Cho S.K., Shin H., Jang I., Park J., Yu K., Cho J. (2015). Antihyperglycemic mechanism of metformin occurs via the AMPK/LXRα/POMC pathway. Scientific Reports; 5:8145. doi:10.1038/srep08145.

Dai M.X., Zheng X.H., Yu J., Yin T., Ma M.J., Zhang L., Liu M., Ma Y., Liu L.W., Gao X., Li Y., Song L.Q., Wang H.C. (2014). The Impact of Intermittent and Repetitive Cold Stress Exposure on Endoplasmic Reticulum Stress and Instability of Atherosclerotic Plaques. Cell Physiol Biochem; 34:393-404. DOI:10.1159/000363008

De Wilde J., Smit E., Mohren R., Boekschoten M.V., De Groot P., van den Berg S.A., Bijland S., Voshol P.J., van Dijk K.W., de Wit N.W., Bunschoten A., Schaart G., Hulshof M.F., Mariman E.C. (2009). An 8-week high-fat diet induces obesity and insulin resistance with small changes in the muscle transcriptome of C57BL/6J mice. J Nutrigenet Nutrigenomics (6):280-91.doi: 10.1159/000308466.

Flatt J.P., Ravussin E., Acheson K.J., Jequier E. (1985). Effects of dietary fat on postprandial substrate oxidation and on carbohydrate and fat balances. J. Clin. Invest.; 76:1019-1024.

Friedewald W.T., Levy R.I., Fredrickson D.S. (1972). Estimation of the concentration of low- density lipoprotein cholesterol in plasma, without the use of preparative centrifuge. Clin. Chem.;18: 499-502.

Gabaldon A.M., Florez-Duquet M.L., Hamilton J.S., McDonald R.B., Horwitz B.A. (1995). Effects of age and gender on brown fat and skeletal muscle metabolic responses to cold in F344 rats. Am J Physiol.; 268:R931–941.

Gasparetti A.L., de Souza C.T., Pereira-da-Silva M., Oliveira R.L.G.S., Saad M.J.A., Carneiro E.M., Velloso L.A. (2003). Cold Exposure Induces Tissue-Specific Modulation of the Insulin-Signalling Pathway in Rattus Norvegicus. J. Physiol.,; 552(Pt 1), 149–162.

Gordon C.J. (2012). Thermal physiology of laboratory mice: Defining thermoneutrality. J Thermal Biol.; 37: 654–685

Hoehn K., Marieb E.N. (2010). Human Anatomy and Physiology. San Francisco: Benjamin Cummings; ISBN 0-321-60261-7.

Ige A.O., Adewoye E.O. (2016). Oral Magnesium Treatment Reduces Anemia and Levels of Inflammatory Markers in Experimental Diabetes. J Diet Suppl.; 26:1-13.

Jermyn M.A. (1975). Increasing the sensitivity of the anthrone method of carbohydrate. Anal. Biochem; 68: 322-335.

Jia Y.J., Liu J., Guo Y.L., Xu R.X., Sun J., Li J.J. (2013). Dyslipidemia in rat fed with high-fat diet is not associated with PCSK9-LDL-receptor pathway but ageing. J Geriatr Cardiol.; 10(4):361-8. doi: 10.3969/j.issn.1671-5411.2013.04.007.

Lee P., Swarbrick M.M., Ho K.K. (2013). Brown adipose tissue in adult humans: a metabolic renaissance. Endocr Rev; 34:413–438

Matthews D.R., Hosker J.P., Rudenski A.S., Naylor B.A., Treacher D.F., Turner R.C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia; 28: 412–419

Moreno-Aliaga M.J., Lamas O., Marti A., Martinez J.A. (2002) Effects of a beta3-adrenergic agonist on glucose uptake and leptin expression and secretion in cultured adipocytes from lean and overweight (cafeteria) rats. Biochem Biophys Res Commun.; 291, 1201–1207.

Moreno-Navarrete J.M., Ortega F.J., Rodríguez-Hermosa J.I., Sabater M., Pardo G., Ricart W., Fernández-Real J.M. (2011). OCT1 Expression in adipocytes could contribute to increased metformin action in obese subjects. Diabetes.; 60(1): 168-76.

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (2014). Insulin resistance and prediabetes. NIH Publication No. 14–4893.

Ohtani N., Sugano T., Ohta M. (1999). Alterations in monoamines and GABA in the ventromedial and paraventricular nuclei of the hypothalamus following cold exposure, a reduction in noradrenaline induces hyperphagia. Brain Res.; 842:6–14.

Ohtsubo K., Chen M.Z., Olefsky J.M., Marth J.D. (2011). Pathway to diabetes through attenuation of pancreatic beta cell glycosylation and glucose transport. Nat. Med. 17, 1067–1075.

Patanè G., Piro S., Rabuazzo A.M., Anello M., Vigneri R., Purrello F. (2000). Metformin restores insulin secretion altered by chronic exposure to free fatty acids or high glucose: a direct metformin effect on pancreatic beta-cells. Diabetes. 49(5): 735-40.

Piro S., Rabuazzo A.M., Renis M., Purrello F. (2012). Effects of metformin on oxidative stress, adenine nucleotides balance, and glucose-induced insulin release impaired by chronic free fatty acids exposure in rat pancreatic islets. Journal of Endocrinological Investigation. 35, (5) 504–510.

Puigserver P., Wu Z., Park C.W., Graves R., Wright M. and Spiegelman B.M. (1998). A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell; 92: 829–839.

Scarpace P.J., Tse C., Matheny M. (1996). Thermoregulation with age, restoration of beta (3)-adrenergic responsiveness in brown adipose tissue by cold exposure. Proc Soc Exp Biol Med; 211, 374–380.

Seifarth C., Schehler B., Schneider H.J. (2013). Effectiveness of metformin on weight loss in non-diabetic individuals with obesity. Exp Clin Endocrinol Diabetes.; 121(1): 27-31. doi: 10.1055/s-0032-1327734.

Seifter S., Dayton S., Novic B., and Muntwyler E. (1950): The estimation of glycogen with the anthrone reagent. Archs. Biochem. 25: 191 – 200.

Smith O.L. (1984). Insulin response in rats acutely exposed to cold. Can J Physiol Pharmacol.; 62:924–927.

Stefanovski D., Richey J.M., Woolcott O., Lottati M., Zheng D., Harrison L.N., Ionut V., Kim S.P., Hsu I., Bergman R.N.(2011). Consistency of the Disposition Index in the Face of Diet Induced Insulin Resistance: Potential Role of FFA. PLoS ONE; 6:e18134. doi:10.1371/journal.pone.0018134

Swinburn B.A., Caterson I., Seidell J.C., James W.P.T. (2004). Diet, nutrition and the prevention of excess weight gain and obesity. Public Health Nutr.; 7(1A): 123–146

Tack C., Pohlmeier H., Behnke T., Schmid V., Grenningloh M., Forst T., Pfutzner A. (2012): Accuracy evaluation of five blood glucose monitoring systems obtained from the pharmacy: A European multicenter study with 453 subjects. Diabetes Technol. Ther.; 14(4): 330-337.

Tahrani A.A., Bailey C.J., Del Prato S., Barnett A.H. (2011). Management of type 2 diabetes: new and future developments in treatment. Lancet. 9; 378(9786): 182-97.

Torsoni M.A., Carvalheira J.B., Pereira-da-Silva M., de Carvalho-Filho M.A., Saad M.J., Velloso L.A. (2003). Molecular and functional resistance to insulin in hypothalamus of rats exposed to cold. Am J Physiol Endocrinol Metab.; 285: E216–223.

Wang X., Che H., Zhang W., Wang J., Ke T., Cao R., Meng S., Li D., Weiming O., Chen J., Luo W. (2015). Effects of Mild Chronic Intermittent Cold Exposure on Rat Organs Int J Biol Sci.; 11(10): 1171–1180.

Warnick G.R., Knopp R.H., Fitzpatrick V., Branson l. (1990). Estimating low density lipoprotein cholesterol by Friedewald equation is adequate for classifying patients on the basis of nationally recommended cut points. Clin. Chem.; 36, 15-19.

Wittert G.A., Or H.K., Livesey J.H., Richards A.M., Donald R.A., Espiner E.A. (1992). Vasopressin, corticotrophin-releasing factor, and pituitary adrenal responses to acute cold stress in normal humans. J Clin Endocrinol Metab; 75(3): 750-755.

Zhukova N.V., Novgorodtseva T.P. and Denisenko Y.K. (2014). Effect of the prolonged high-fat diet on the fatty acid metabolism in rat blood and liver. Lipids in Health and Dis.; 13:49 DOI: 10.1186/1476-511X-13-49