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CASE REPORT

Intestinal Obstruction Secondary to an Intra-Abdominal Foreign Body

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According to literature, the incidence of intestinal obstruction caused by internal abdominal hernia is very rare and has an occurrence rate of about 0.2-0.9%. Internal hernias are caused by defects that occur congenitally or as a result of surgery or trauma. It is still rarer for surgical instruments inadvertently left in the abdominal cavity after laparotomy to be the cause of internal herniation resulting in intestinal obstruction. A case of intestinal obstruction caused by an artery forceps left in the abdominal cavity after surgery is presented.

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Keywords: Internal hernia, foreign body, artery forceps, adhesions, intestinal obstruction

INTRODUCTION

From literature, it is known that intestinal obstruction caused by internal abdominal hernias is rare, with incidence rates of 0.2 – 0.9% at autopsy (Ghahremani, 1984) out of which about 0.5 – 5.8% are due to internal herniation (Pershad *et al.*, 1998). Internal abdominal hernias arise as a result of congenital defects due to anomalies of mesenteric fixation and intestinal rotation during foetal development or as a result of surgery or trauma (Yagmik *et al.*, 2009; Mathieu and Lucian, 2004). It is uncommon to have foreign bodies (e.g. surgical instruments) left behind in the abdominal cavity following laparotomy causing herniation and intestinal obstruction (Pershad *et al.*, 1998). Foreign materials, including surgical instruments and sponges left in the peritoneal cavity after laparotomy, are potentially dangerous medical errors (Lincourt, 2007). Retention of surgical instruments and materials in the abdominal cavity is uncommon because it is under-reported and can carry serious medico-legal consequences (Karahasanoglu *et al.*, 2004; Berkowitz *et al.*, 2007; Ugochukwu and Edeh, 2011). Foreign bodies

inadvertently retained in the abdominal cavity range from small gauzes and sponges (referred to as gossypiboma) to artery and tissue forceps, scissors, retractors, needles, spatulas and others (AORN, 2006; Wan *et al.*, 2009; Gibbs, 2011). Adhesions forming around these foreign bodies (gauzes and sponges) usually lead to intestinal obstruction (Lauwers and Hee, 2000). In the case of instruments, they are usually inert and can only cause intestinal obstruction if they compress a section of the bowel or the bowel is caught in the jaws of the instrument (Ugochukwu and Edeh, 2011). A case of intestinal obstruction as an outcome of an artery forceps being inadvertently left in the peritoneal cavity thus resulting in intestinal obstruction due to herniation of the small intestine through the handle/finger loop of the artery forceps is reported in this study.

CASE REPORT

A 39-year-old woman presented with abdominal pain of 3 days and constipation of 2 days duration. The pain was aching in nature, constant, centrally located and so severe she had to stop all activity. There were no relieving or aggravating factors. This was followed by absolute constipation, anorexia, vomiting and fever.

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She had surgery done in one of the hospitals in the Kumasi Metropolis three and a half months prior to presentation on account of a ruptured ectopic gestation. She was haemo-transfused during and after the surgery and was discharged a week later. Eight weeks after discharge she reported to her doctors with intermittent colicky pains, vomiting and constipation. She was seen on several occasions with the same complaints and was always given analgesics and sent back home. When the pains became constant, associated with vomiting and constipation she decided to report to Komfo Anokye Teaching Hospital (KATH) for further management.

Physical examination revealed a middle-aged woman with vital signs (including temperature, blood pressure and pulse rate) being normal. The abdomen appeared full with the presence of a Pfannenstiel incisional scar from the previous surgery and tender on palpation with guarding. The hernia orifices were intact and no bowel sounds were heard on auscultation. Digital rectal examination yielded normal findings. Her white blood cell count was $14.3 \times 10^9/L$ and the renal function tests were normal. A chest radiograph showed no air under the diaphragm but a plain erect abdominal radiograph showed air-fluid levels with the outline of a metal instrument that looked like an artery forceps (Figure 1). A diagnosis of intestinal obstruction secondary to an intra-abdominal foreign body (artery forceps) was made and the patient prepared for laparotomy.

At laparotomy, a loop of small bowel was found to have herniated through one handle/finger loop of the artery forceps at a distance of about 24cm from the ileo-caecal junction (Figure 2). This loop of bowel, measuring about 40 cm in length, was totally gangrenous (Figure 3). The artery forceps was otherwise lying freely in the peritoneal cavity without any adhesions. There were also no adhesions between the bowel loops. The gangrenous small bowel was resected en-bloc with the artery forceps and an end-to-end anastomosis done to restore bowel continuity. The post-operative period was uneventful and the patient was discharged to go home on the 5th post-operative day in a satisfactory condition. She was

subsequently reviewed on two occasions after discharge and had no complaints on both occasions.

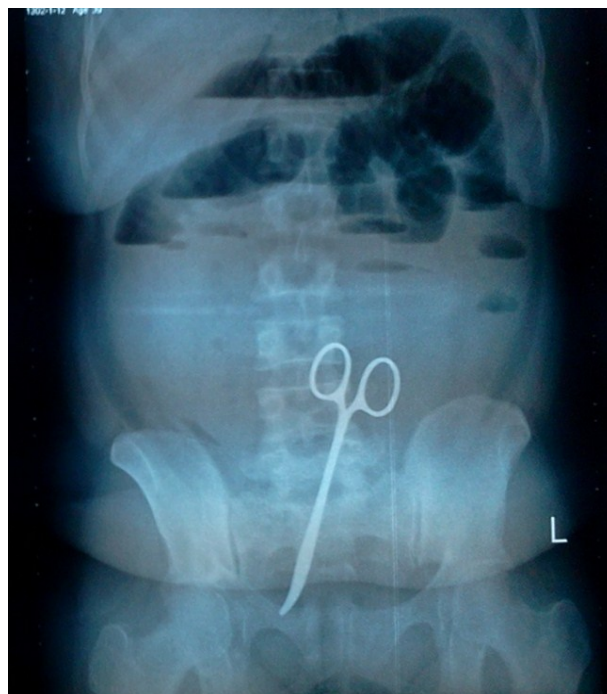


Figure 1: Plain erect abdominal radiograph showing an artery forceps in the peritoneal cavity. Also seen are air-fluid levels.



Figure 2: Artery forceps with the bowel herniating through one of the handle/finger loops



Figure 3: Herniation of loop of bowel through the handle loop, another view.

DISCUSSION

Even though there is paucity of data on retained surgical instruments and material in the sub-region, the problem is real and must be tackled holistically. The surgical team (surgeon, anaesthetist, scrub nurse, nurse runners and anyone involved in the operation) must be conversant with the risk factors associated with retention of surgical items during operating procedures.

Inadvertently retained surgical instruments or materials in the abdominal cavity are an uncommon but dangerous surgical error usually occurring during laparotomy (Gawande *et al.*, 2003). It is a serious and embarrassing occurrence in surgical practice and as such is under-reported and under-estimated (Asuquo *et al.*, 2006) probably due to medico-legal implications, the unwillingness of surgeons to publicize such errors and/or the complacency of colleagues in exposing the occurrence for fear of jeopardizing a professional life (Uguchukwu and Edeh, 2011). These retained materials and instruments in the peritoneal cavity can go undetected for years if they cause no problems and are usually found accidentally when the patient is being investigated for a different condition altogether (Nasir, 2009). The presence

of foreign bodies in the abdominal cavity can lead to the formation of adhesions which are a common cause of small bowel obstruction in post-operative patients (Lauwers and Hee, 2000). Such materials or instruments can cause infection of the peritoneal cavity and perforations of hollow viscera and may result in severe morbidity or even mortality if measures are not taken to diagnose and remove the offending instrument or material (Lauwers and Hee, 2000; Kalovidouris *et al.*, 1999).

Foreign bodies unintentionally retained in the abdominal cavity include towels, artery forceps, pieces of broken instruments or irrigation sets and rubber tubes (Garg and Agarwal, 2010). The most common of foreign bodies left in the abdomen are small surgical sponges and towels, usually referred to as gossypibomas or textilomas (Rapaport and Haynes, 1990; Yildiririm *et al.*, 2006). Several studies have been conducted to identify the risk factors for surgical material retention in patients after surgery and the symptoms caused by these materials. The three main risk factors for retention of a foreign body in the abdominal cavity after multivariate analysis of many factors include: emergency surgeries, unplanned changes in surgical procedure, and a higher body mass index (Gawande *et al.*, 2003). Patients with high BMI are likely to have large greater omentum hence the likelihood of a foreign body hiding underneath without being noticed. This patient had emergency surgery for a ruptured ectopic pregnancy and was obese (BMI of 35.2 kg m²). She therefore had two out of the three identified risk factors for the retention of surgical instruments or material in the abdomen after laparotomy.

Other risk factors considered in literature which can lead to retention of surgical material after laparotomy include: lengthy surgical procedures, change in nursing staff during the procedure, poor communication among the operating team, operations performed late at night, more than one surgical team being involved in the operation, inexperienced and inadequate staff, staff fatigue, performance of a major procedure, unstable patient condition, the necessity to arrest massive intra-abdominal bleeding using multiple instruments and

packs of gauze, improper lighting in the theatre, hurried or non-meticulous sponge and instrument count, as well as absence of the surgeon at the time of wound closure (Murad and Basi, 2003; Wang *et al.*, 2009; Dakubo *et al.*, 2009). Knowledge of such risk factors is important to forestall unintentional leaving of surgical material and instruments after abdominal surgery. It is imperative that extreme care is taken during the performance of simple but vital tasks, such as counting of instruments and gauze to prevent them being left behind resulting in complications to the patient and cost to clinical practice in terms of law suits, morbidity and even mortality of patients.

To date, the only two documented reports of surgical material being left in the abdominal cavity from literature in Ghana are from the Korle-Bu Teaching Hospital in Accra (Dakubo *et al.*, 2009; Adu-Aryee *et al.*, 2005). But for anecdotal accounts by surgeons to colleagues, there is no confirmation of any reports of retained surgical instruments or material in the abdominal cavity from Komfo Anokye Teaching Hospital, Kumasi, Ghana as such making this case report; the first to be reported from KATH. On the backdrop of evidence from available literature, surgeons need to have a high index of suspicion and consider retained surgical instruments or material, if after surgery; a patient has vague, non-specific abdominal signs and symptoms.

The symptomatology of retained foreign material, in the abdominal cavity gleaned from the world scientific literature reflects paucity of clinical signs and symptoms. The symptoms are usually non-specific and varied during the post-operative period until an emergency condition such as intestinal obstruction occurs as a result of adhesions caused by the retained surgical item. Wan *et al.*, (2009) reported such signs and symptoms to include: vague abdominal pains or irritation, palpable mass, anorexia, weight loss, fatigue, fever, nausea, vomiting, rectal bleeding and so forth which are non-specific. This patient had abdominal pains and so was seen and treated with analgesics for several weeks before she decided to seek medical care at KATH when she developed vomiting and constipation in addition.

CONCLUSION

It is highly imperative that the surgical team sticks strictly to theatre etiquette of counting surgical material several times: once before starting the procedure, during the procedure, before the abdominal cavity is closed and at the end of the procedure as recommended by AORN, (2006). Furthermore, for cases identified as high risk, additional preventive measures should be considered as this will go a long way in minimizing retention of surgical items in the abdominal cavity, if not eliminating it altogether. However, for diagnosis of a retained surgical item to be made in a patient there should be a high index of suspicion from the part of the surgeon and not an over-reliance on any specific symptoms.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Effect of Xylopic Acid on Paclitaxel-induced Neuropathic pain in rats

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Xylopic acid, a diterpenoid isolated from the fruits of *Xylopic acid*, a diterpenoid isolated from the fruits of *Xylopic acid* has demonstrated analgesic properties in acute pain models. It was therefore evaluated for its analgesic properties in paclitaxel-induced neuropathic pain, a type of pain difficult to treat clinically. Neuropathic pain was induced in rats by injecting 2 mg kg⁻¹ of paclitaxel on alternative days for four days (days 0, 2, 4 and 6). Paclitaxel-induced cold allodynia, mechanical hyperalgesia and thermal hyperalgesia were measured during pre-paclitaxel administration and on day 16 post-paclitaxel administration. The rats were treated with xylopic acid (10, 30 and 100 mg kg⁻¹; groups 1-3), pregabalin (10, 30 and 100 mg kg⁻¹; groups 4-6) and vehicle (group 7) daily for 5 days. Pain thresholds were also measured daily for 5 days in the three models. Xylopic acid and pregabalin produced analgesic properties seen as increased paw withdrawal latencies to mechanical and cold water stimuli during the five days treatment. In addition, the two agents significantly (P<0.05) exhibited analgesic properties in the thermal hyperalgesia test. These data suggest that xylopic acid is an effective agent against paclitaxel-induced neuropathic pain.

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Keywords: Xylopic acid, paclitaxel, neuropathic, pregabalin, hyperalgesia, cold allodynia

INTRODUCTION

It is estimated that, more than half of patients with cancer are treated with chemotherapeutic agents such as taxanes (paclitaxel), platinum-based compounds and vinca alkaloids and about 40% of such patients are prone to neuropathic pain (Deng *et al.*, 2012). The incidence and severity of paclitaxel-induced neuropathic pain symptoms correlates with increasing cumulative doses of paclitaxel (Akerley *et al.*, 1998; Postma *et al.*, 1995). Paclitaxel, an anti-cancer drug was originally derived from the bark of the Western yew tree, *Taxus brevifolia*. It is used to treat several tumours including ovarian, breast and lung cancers. The antineoplastic activity of paclitaxel is thought to

involve disruption of microtubule assembly; an important cellular component responsible for development and maintenance of neurons, mediation of axonal transport in the neurons and provision of structural support for neurons (Bray *et al.*, 1988; Kobayashi and Mundel, 1998). The most common clinical neurotoxicity associated with the use of paclitaxel is sensory peripheral neuropathy which is often dose-related and may begin as early as 24-72 hours after administration of high single dose of paclitaxel (Rowinsky *et al.*, 1993). Patients describe various sensory symptoms including mechanical allodynia, spontaneous pain, cold allodynia, numbness and tingling (Rowinsky *et al.*, 1993; Forsyth *et al.*, 1997; Dougherty *et al.*, 2004).

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In an attempt to solve this enigma, several plants and isolated compounds from them have been tested against paclitaxel-induced neuropathic pain. Eth-

anolic fruit extract of *Xylopic aethiopic* has been shown to possess anti-tumour properties (Adaramoye *et al.*, 2011). Xylopic acid, a major diterpene isolated from the fruits of *X. aethiopic* is devoid of anticancer properties but has shown antinociceptive properties in several animal models of pain (Cavalcanti *et al.*, 2009; Woode *et al.*, 2012). It is against this backdrop that the analgesic property of xylopic acid was evaluated in paclitaxel-induced neuropathic pain in rats.

MATERIALS AND METHODS

Experimental animals and housing

Sprague-Dawley rats (200–250 g) of both sexes were housed in stainless steel cages (n=5) for a week in the laboratory to acclimatize with the environment. The animals were fed with normal commercial pellet diet (AGRICCARE, Kumasi) and water *ad libitum* and kept under standard laboratory conditions. All experiments were performed during the day between the hours of 8:00–15:00.

The procedures and techniques used in the studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, 1985, revised 1996). All protocols used were approved by the Departmental Ethics Committee.

Drugs and Chemicals

Pregabalin (Lyrica®) was purchased from Pfizer Pharmaceuticals (Arzneimittelwerk Godecke, Freiburg, Germany), cremophor from Sigma-Aldrich Inc. (St. Louis, MO, USA) and paclitaxel (Intaxel®) from Fresenius Kabi Oncology (Badi, India).

Extraction and purification of xylopic acid ((15- β -Acetoxy-(-)-kaur-16-en-19-oic Acid)

Xylopic acid was extracted according to the process described by Woode *et al.*, (2012). Briefly, 360 mg of the fruit of *Xylopic aethiopic* was macerated with 5 L of petroleum ether (40–60 °C) and allowed to stand for three days. The petroleum ether was drained and concentrated with rotary evaporator at a temperature of 50°C. To facilitate crystallization of xylopic acid, ethyl acetate was added to the concentrate. Crystals (xylopic acid) formed after three days were washed

with petroleum ether at 40–60°C. Crude xylopic acid was purified in 96% ethanol. The yield of the xylopic acid was 1.41%. The purity of the isolated xylopic acid was 95% with high performance liquid chromatography.

Paclitaxel Administration

Rats were allowed to acclimatize to the behavioural testing environment and baseline measurements of mechanical, thermal and cold stimuli were performed. Neuropathic pain was induced in the rats by intraperitoneal (i.p.) injection of paclitaxel (2 mg kg⁻¹) dissolved in saline on four alternate days (days 0, 2, 4 and 6) as described by Ameyaw *et al.*, (2013); Flatters and Bennett, (2004). On day 16 post-paclitaxel treatments, xylopic acid (10, 30 and 100 mg kg⁻¹ dissolved in cremophor; groups 1-3), pregabalin (10, 30 and 100 mg kg⁻¹; groups 4-6) and cremophore solution (group 7) were administered to the rats after confirmation of neuropathic pain in the various tests. The effect of xylopic acid, pregabalin and cremophor treatments on paclitaxel-induced neuropathic pain were evaluated in the Randall-Sellitto paw pressure-, thermal tail immersion- and cold- allodynia tests.

Behavioural assessment of neuropathic pain

Mechanical hypersensitivity

The effect of xylopic acid (10-100 mg kg⁻¹), pregabalin (10-100 mg kg⁻¹) and cremophor solution on mechanical hyperalgesia was measured with the Randall-Sellitto paw pressure analgesimeter (IITC Life Science Model 2888 Woodland Hills, CA, USA) as previously described by Woode *et al.*, (2012). The rat's hind paw was placed into a pressure applicator, and a steadily increasing pressure stimulus (maximum cut-off of 250 g) was applied to the dorsal surface of the paw until withdrawal or vocalization. This was recorded as the nociceptive threshold value. For each animal, two recordings were made for each hind paw, and the data were reported as the mean of both hind paw values.

Thermal Hyperalgesia

The tail immersion test was used to determine the effect of xylopic acid (10-100 mg kg⁻¹), pregabalin (10-100 mg kg⁻¹) and cremophore solution on ther-

mal hyperalgesia (Thirumal *et al.*, 2013). The distal portion of the tail (3 - 4 cm) of the rat was immersed in hot water maintained at 52°C temperature until the tail was withdrawn. The duration of immersion was recorded and a cut-off time of 10 s was used.

Cold allodynia

The analgesic effect of xylopic acid (10-100 mg kg⁻¹), pregabalin (10-100 mg kg⁻¹) and cremophor solution on cold allodynia was assessed by immersing the rat's hind paw into cold water (4.5°C). The latency for a rat to withdraw its paw was measured with a digital timer as described by Kim *et al.*, (2005). Only one hind paw was assessed during each immersion at a time with a cut-off time of 20 s. For each animal, two recordings were made for each hind paw, and the withdrawal responses were reported as the mean of both hind paw values.

Statistical analysis

Data were analyzed with GraphPad Prism Version 5 (GraphPad Software, San Diego, CA, USA). The results are presented as mean ± S.E.M. The time-course curves were subjected to two-way (treatment × time) repeated measures of analysis of variance (ANOVA) with Bonferroni's *post hoc* test. Doses for 50% of the maximal effect (ED₅₀) for each drug were determined by using an iterative computer least squares method, with the following nonlinear regression (three-parameter logistic) equation:

$$Y = \frac{a + (b - a)}{1 + 10^{(LogED_{50} - X)}}$$

Where, X is the logarithm of dose and Y is the response. Y starts at a (the bottom) and goes to b (the top) with a sigmoid shape.

The fitted midpoints (ED₅₀) of the curves were compared statistically using F-test (Miller, 2003; Motulsky and Christopoulos, 2003). ED₅₀ determinations were also done with GraphPad Prism Version 5. For all comparisons, a P < 0.05 was considered statistically significant.

RESULTS

Injection of a cumulative dose of 8 mg kg⁻¹ of

paclitaxel into rats produced neuropathic pain that lasted weeks after the injection. Neuropathic pain was confirmed in the mechanical hyperalgesia, cold allodynia and thermal hyperalgesia models on the 16th day post paclitaxel injection. Xylopic acid (10-100 mg kg⁻¹) produced significant (P<0.0001) analgesic properties in the Randall-Sellito test (Figure 1A). Treatment of rats with 100 mg kg⁻¹ xylopic acid reversed the mechanical hyperalgesia significantly from day two to five. The 10 and 30 mg kg⁻¹ xylopic acid treatments significantly reversed the mechanical hyperalgesia except for day two. Pregabalin (10-100 mg kg⁻¹) similarly produced analgesic properties in this model (Figure 1B). The potency of xylopic acid in this model was 2.54 times the potency of pregabalin (Table 1).

A sustained thermal hyperalgesia was observed in the control rats but not animals treated with xylopic acid and pregabalin. Xylopic acid at doses of 100 and 30 mg kg⁻¹ reduced significantly the thermal hyperalgesia during the five days daily treatments (Figure 2A). On the contrary, the lowest dose of xylopic acid did not produce any significant thermal

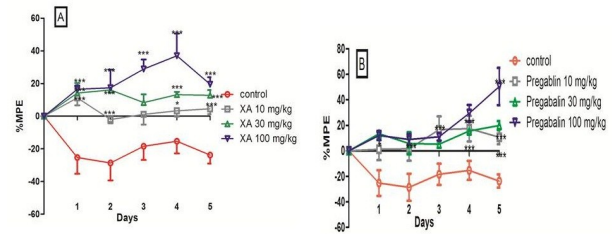


Figure 1: The time-course curve of the effects of daily dosing of xylopic acid (10-100 mg/kg), pregabalin (10-100 mg/kg) and cremophor solution (control) on established paclitaxel-induced mechanical hyperalgesia. Graph A shows the effect of daily systemic administration of 10-100 mg/kg xylopic acid or vehicle and B shows daily systemic administration of 10-100 mg/kg pregabalin or vehicle for five days. Each point represents Mean ± S.E.M (n = 5); *P ≤ 0.05, **P ≤ 0.01, *P ≤ 0.001, compared to respective controls (Two-way repeated measures ANOVA followed by Bonferroni's post hoc).**

hyperalgesia at any time point. Pregabalin, similar to xylopic acid produced significant ($P < 0.0001$) reversal of thermal hyperalgesia (Figure 2B). Xylopic acid was 4.3 times more potent than pregabalin (Table 1) in the thermal hyperalgesia test.

The latency to paw withdrawal to cold stimulus was significantly prolonged after treating the animals with xylopic acid (10-100 mg kg⁻¹; Figure 3A) and pregabalin (10-100 mg kg⁻¹; Figure 3B) compared to vehicle treated animals. The analgesic effect of pregabalin was significant at all the time points and dose-dependent. In this model, xylopic acid was 2.4 times potent than pregabalin (Table 1).

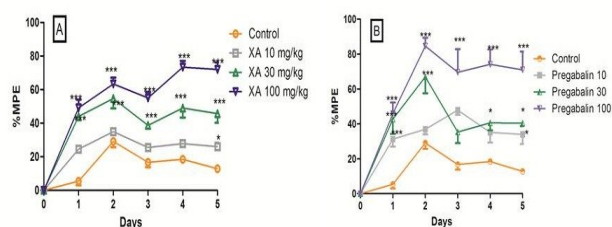


Figure 2: The time-course curve of the effects of daily dosing of xylopic acid (10-100 mg kg⁻¹), pregabalin (10-100 mg kg⁻¹) and cremophor solution (control) on established paclitaxel-induced thermal hyperalgesia. Graph A shows the effect of daily systemic administration of 10-100 mg kg⁻¹ xylopic acid or vehicle and B shows daily systemic administration of 10-100 mg kg⁻¹ pregabalin or vehicle for five days. Each point represents Mean \pm S.E.M (n = 5); *P \leq 0.05, **P \leq 0.01, *P \leq 0.001, compared to respective controls (Two-way repeated measures ANOVA followed by Bonferroni's post hoc).**

Table 1: Respective ED₅₀ (mg kg⁻¹ \pm S.E.M.) for xylopic acid and pregabalin in cold allodynia, mechanical and thermal hyperalgesia tests

Test	Pregabalin	Xylopic acid
Mechanical hyperalgesia (Randall-Sellito test)	7.18 \pm 0.51	18.21 \pm 0.38
Thermal hyperalgesia (Tail immersion test)	4.32 \pm 0.96	16.09 \pm 0.95
Cold allodynia	8.1 \pm 0.99	19.33 \pm 0.85

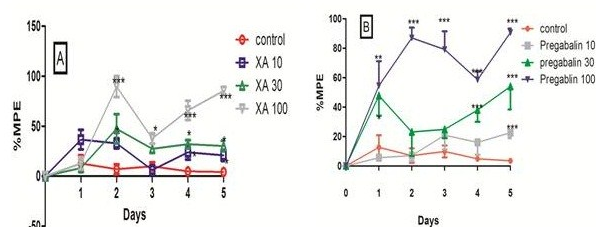


Figure 3: The time-course curve of the effects of daily dosing of xylopic acid (10-100 mg kg⁻¹), pregabalin (10-100 mg kg⁻¹) and cremophor solution (control) on established paclitaxel-induced cold allodynia. Graph A shows the effect of daily systemic administration of 10-100 mg kg⁻¹ xylopic acid or vehicle and B shows daily systemic administration of 10-100 mg kg⁻¹ pregabalin or vehicle for five days. Each point represents Mean \pm S.E.M (n = 5); *P \leq 0.05, **P \leq 0.01, *P \leq 0.001, compared to respective controls (Two-way repeated measures ANOVA followed by Bonferroni's post hoc).**

DISCUSSION

Neuropathic pain induced with a cumulative dose of 8 mg kg⁻¹ paclitaxel administered in four injections resulted in significant cold allodynia, mechanical and thermal hyperalgesia. Xylopic acid and pregabalin, the standard drug, inhibited the hyperalgesia associated with thermal and mechanical stimulation as well as the cold allodynia associated with cold water stimulus. Pharmacokinetically, paclitaxel formulated as Cremophor-ethanol (Taxol) preparation distributes in the central and peripheral nervous system in rats following its administration (Cavaletti *et al.*, 2000). Paclitaxel accumulates in the dorsal root ganglia and the brain at very low concentrations. Accumulation has been reported in the sciatic nerve and spinal cord at intermediate concentrations (Cavaletti *et al.*, 2000).

The neuropathy in this study after low dose paclitaxel administration was due to atypical (swollen and vacuolated) mitochondria in peripheral sensory axons—both C-fiber and myelinated axons and a loss of intra-epidermal nerve fibres (Fidanboyu *et al.*, 2011). Allodynia caused by paclitaxel neurotoxicity is as a result of apoptosis in the dorsal root ganglion neurons (Seong *et al.*,

2013). Paclitaxel induces morphological changes (swollen and vacuolated mitochondria) and dysfunction (reduced respiration and energy production) of mitochondria in axons, which then alters intracellular calcium levels and initiates apoptotic pathways (Flatters *et al.*, 2006; Melli *et al.*, 2008; Xiao *et al.*, 2011; Zheng *et al.*, 2011). The exact mechanism of xylopic acid in this model cannot be pointed out but it is likely that as a calcium channel antagonist (Somova *et al.*, 2001), it inhibited calcium channels to stabilize the nerve membrane. Pregabalin is effective both experimentally and clinically in the management of neuropathic pain. Its action is as a result of antagonist effect on $\alpha^{2-\delta 1}$ Ca²⁺ channel subunit of N-type voltage dependent calcium channels. Inhibition of calcium channels prevent neuronal excitability and other cellular enzymatic cascade reactions that lead to pain sensation (Schim, 2009; Kumar *et al.*, 2010).

The effect of xylopic acid on pro-inflammatory pain mediators and cytokines cannot be ruled out. Several reports indicate that paclitaxel evokes pro-inflammatory pain mediators and cytokines, including bradykinin and TNF- α as well as the activation of microglial and astroglial cells (Costa *et al.*, 2011; Burkhart *et al.*, 1994; Manthey *et al.*, 1992; Zhang *et al.*, 2012; Burgos *et al.*, 2012). It has been reported that xylopic acid inhibits the nociceptive effects of bradykinin and glutamate (Woode *et al.*, 2013). The blocking of the effects of these pain mediators may contribute to the observed analgesic properties in the mechanical and thermal hyperalgesia as well as cold allodynia tests. Glutamatergic neurotransmission and N-methyl-D-aspartate (NMDA) receptors are involved in paclitaxel-induced neuropathic pain (Jaggi *et al.*, 2012). Peripheral nerve damage results in glutamate/NMDA receptor-mediated sensitization and spontaneous activity of primary afferents, and causes hyper-excitability of dorsal horn neurons and down-regulation of glial glutamate transporters (i.e. GLAST and GLT-1) in the spinal dorsal horn (Petrenko *et al.*, 2003; Zhang *et al.*, 2012).

In addition, xylopic acid suppresses pain via the opioidergic nociceptive pathway (Woode *et al.*, 2013) and this may partly contribute to the analgesic properties of xylopic acid in this model. Agents such as

morphine that blocks the opioidergic nociceptive pathway have been shown to inhibit paclitaxel-induced neuropathic pain (Ami *et al.*, 2012).

CONCLUSIONS

The data presented indicate that xylopic acid exerts analgesic properties in paclitaxel-induced neuropathic pain in rats and may be useful in managing neuropathic pain associated with chemotherapy in man.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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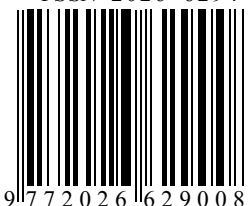
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ORIGINAL ARTICLE

Prevalence and Risk Factors for Overweight and Obesity among Nurses in the Tamale Metropolis of Ghana

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Prevalence of overweight and obesity is increasing in various populations, and is becoming a huge problem among occupational/professional groups that are perceived as preponderantly sedentary. An attendant acquisition or imposition of a lowered physical activity level and other lifestyle with such occupations may contribute to the development of obesity and overweight. The objectives of the study were to determine the prevalence of obesity and overweight among nurses using Body Mass Indexes (BMI) and waist-to-hip ratios (WHR), to assess dietary habits, ascertain daily lifestyles in terms of physical activity and nutrition and to determine the associations between age, marital status, dietary habit, physical activity and BMI/WHR. The study was conducted in the four main hospitals within the Tamale metropolis of the Northern region of Ghana. A cross sectional analytical design was used for the study. Two hundred and twenty (220) nurses were selected based on a probability proportionate to size (PPS). A structured questionnaire was used as the instrument for data collection and both qualitative and quantitative data were collected and analysed statistically using SPSS. The ages of respondents ranged from 20 to 60 years. The 20-30 years age group had the highest number of respondents (67.3%) with the age group of 41-50 having the lowest number (5.0%). In terms of gender, females dominated with 146 respondents representing 66.4% and males were 74 representing 33.6%. One hundred and nineteen (54.0%) of the respondents were married whilst 92 (41.8%) were never married, 6 (2.7%) were widowed and 3 (1.4%) were divorced in that order. The prevalence of overweight and obesity among the nurses were 26.4% and 16.9% respectively. Physical inactivity and dietary habit especially skipping of meals was found to be contributing factors to overweight and obesity among the nurses. Age, gender and marital status had an influence on the level of obesity and overweight among the nurses as the older nurses were more likely to be obese than the younger ones, female nurses were significantly more likely to be obese than the males, whilst those married had a higher tendency to be overweight and obese than the never married, divorced and widowed respectively. Prevalence of overweight and obesity among nurses in the Tamale metropolis is high and of public health significance. Lifestyle and eating habits associated with the nature of the occupation, especially skipping of meals and a predominance of physical inactivity may be significant contributors to the high prevalence of obesity and overweight among the nurses.

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Keywords: Obesity, overweight, lifestyle, nurses, Tamale, Ghana

INTRODUCTION

Overweight and obesity refers to an abnormal or excessive fat accumulation in the body that presents

a risk to an individual's health. Overweight and obesity constitute major risk factors for a number of chronic diseases, including diabetes, cardiovascular diseases and cancer, and various other physical, psychological, and social morbidities as well as depression, discrimination and weight-related bias (Rockville, 2001; Candib, 2007). Excessive fat accumulation in the body tends to increase the risk of

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high blood pressure, high cholesterol, asthma, arthritis, and brings about a general poor health status (WHO, 2000; Centers for Disease Control and Prevention, 2005).

Although the problem of obesity and overweight was once an issue only in high income countries, its prevalence has now drastically risen in low- and middle-income countries that are now facing a "double burden" of disease (Popkin, 1994; 1997; Prentice, 2005; Akpa and Meta, 2008; Abubakari *et al.*, 2008). While many low and middle income countries continue to deal with the problems of infectious disease and under-nutrition, they are also experiencing a rapid upsurge in chronic disease risk factors, which includes obesity and overweight, particularly in urban settings (WHO, 2005; Akpa and Meta, 2008).

The plethora of factors that may account for the growing global epidemic of overweight and obesity include genetics and social factors such as socio-economic status, race/ethnicity, media and marketing, and the physical environment, which influences energy consumption and expenditure (Bouchard *et al.*, 2003; Fezeu *et al.*, 2006; Christensen *et al.*, 2008). The nutritional and socio-economic transition that is occurring in much of the developing world may have contributed to the upsurge in overweight and obesity (Popkin, 1994; 1997; Prentice, 2005). However, overweight and obesity is generally caused by a lack of physical activity, unhealthy eating patterns resulting in excess energy intake, or a combination of the two (Flegal *et al.*, 2002).

Physical inactivity and increased sedentary nature of daily activities have become serious threats to the body as they increase the risk of overweight and obesity, which may be harmful to normal body function and job productivity (Ogunjimi *et al.*, 2010). The quest for ways to make life easier and more comfortable, that is, from the perspective of conserving efforts and human energy through the use of labour saving devices and the disdain for sweating may add to the increasing tendency of overweight and obesity. Technological advancements in the sciences have simplified life and work for many professionals (including health professionals) bringing about a re-

duction in physical activity levels for many individuals in such professions. The result of a reduction in energy expenditure may have implications for overweight and obesity if individuals are exposed to such conditions over a long period.

Anecdotal evidence has shown that many health professionals, especially female nurses, in Ghana have a tendency to be overweight or obese (Health Foundation of Ghana, 2009). In Nigeria (Ogunjimi *et al.*, 2010) and the United States of America (Miller *et al.*, 2008), studies have revealed high prevalence of overweight and obesity among nurses. In Nigeria, for instance, prevalence of overweight and obesity among nurses was found to be 62.2% whereas in the USA it was 54%. Such high prevalence rates, as observed in the two studies may suggest that our healthcare professionals are at an increased risk for various non-communicable diseases (NCD), and if nothing is done to halt this trend then sooner than later our care-givers may become major care receivers (Ogunjimi *et al.*, 2010). According to the studies by Ogunjimi *et al.*, (2010) and Miller *et al.*, (2008) the health and healthcare implications of weight gain are clear for most nurses who are well informed about this menace. It is imperative to investigate the prevalence and potential determinants of obesity and overweight among nurses in Ghana as this would serve as a source of information for health policy formulation in the management of overweight and obesity among health professionals.

MATERIALS AND METHODS

The study is a cross-sectional analytical design where both exposure (nutrition and other lifestyle variables) and outcome (anthropometric variables) were measured simultaneously. A sample size of 220 nurses was selected from a sample frame of 749. Respondents were selected from the four main hospitals within the Tamale metropolis; Tamale Teaching hospital, West and Central hospitals and Seventh Day Adventist (SDA) hospital using a probability proportional to size (PPS). Respondents who were 18 years and above, non-pregnant (females only) and willing to participate were included in the study.

A structured questionnaire was used to collect data on socio-demographic characteristics, nutrition and other lifestyle (physical activity levels) as well as anthropometry. Anthropometric measurements of weight were taken to the nearest 0.1kg using an electronic scale (Uniscale, UNICEF 2008) whilst height, waist and hip circumferences were taken to the nearest 0.1cm using a microtoise (wall-mount retractable non-stretch tape) and a simple non-stretch tape respectively. Body mass index (BMI) was expressed as weight in kg/height in m². Waist-to-Hip ratio (WHR) was derived from the waist and hip circumference measurements. Using WHO (2006) classifications overweight and obesity were defined by BMI 25 to <30 for overweight and ³30 for obesity, and for central obesity a WHR of >0.90 (Males) and >0.80 (Females) depicted a significant risk.

Statistical Analysis

Data was analyzed using SPSS (version 17.0, SPSS Inc., USA). Continuous variables were expressed as means ± SEM whilst categorical variables were ex-

pressed as proportions and/or percentages. Levels of association were determined using Chi-square and linear regression analyses, and a p<0.05 was set as the level at which differences were accepted as being statistically significant.

RESULTS

Socio-demographic characteristics of respondent nurses

The socio-demographic characteristics of the nurses tested for association by gender using Chi-square test as shown in Table 1 (with p-values and difference of margin – phi, φ). From Table 1, it is evident that there was no significant association between gender, religion and ethnicity (p>0.05). However, marital status and age grouping showed a significant association with gender (p<0.001)implying that females were significantly more likely to be older and married compared to their male counterparts.

Nurses of Akan origin were appreciably more compared to the other northern tribes aside Dagombas-

Table 1: The socio-demographic characteristics of respondent nurses stratified by gender

Variable	Male (N=74)	Female (N=146)	Total	Chi-square (χ ²)	
Marital status					
Never married	51 (68.9%)	41 (28.1%)	92 (41.8%)	p<0.00, φ=0.42	
Married	23 (31.1%)	96 (65.7%)	119 (54.0%)		
Divorced	0 (0.0%)	3 (2.1%)	3 (1.4%)		
Widowed	0 (0.0%)	6 (4.2%)	6 (2.7%)		
Religion					
Christianity	36 (48.5%)	85 (58.2%)	121 (55.0%)	p=0.18, φ=0.91	
Islam	38 (51.5%)	61 (41.8%)	99 (45.0%)		
Ethnicity					
Dagombas	38 (51.4%)	64 (43.8%)	102 (46.4%)	p=0.16,φ=0.19	
Akans	14 (18.9%)	26 (17.8%)	40 (18.2%)		
Frafras	8 (10.8%)	26 (17.8%)	34 (15.4%)		
Dagaos	6 (8.1%)	18 (12.3%)	24 (10.9%)		
Kokombas	7 (9.5%)	5 (3.4%)	12 (5.5%)		
Others	1 (1.4%)	7 (4.8)	8 (3.7%)		
Age group (years)					
20-30	63 (85.1%)	85 (58.2%)	148 (67.2%)		p<0.00, φ =0.31
31-40	7 (9.4%)	16 (10.9%)	23 (10.5%)		
41-50	3 (4.1%)	8 (5.5%)	11 (4.9%)		
51-60	1 (1.4%)	37 (25.3%)	38 (17.3%)		

Φ means difference of margins

giving credence to the fact that the Tamale metropolis is rapidly urbanizing and therefore has become a melting pot for many ethnic groupings to settle and work thus reflecting the relatively larger proportion of Akan migrants within the metropolis. With regards to age distribution, higher percentages (67.3%) of nurses fell within the 20-30 years age group with the 41-50 years age group making up (5.0%) of the respondent population. The large number of younger nurses may reflect the enrolment into the study of a relatively large number of freshly graduated nurses doing their rotation within the urban hospitals as opposed to the fewer middle aged nurses (31-40 and 41-50 years) who usually move on after further training. The relatively larger number of 51-60 year olds over the middle aged nurses could probably be explained by the retention of older and more experienced staff (especially midwives) or the preference of older nurses to remain in the big hospitals as they reach their retirement age (Table 1).

Determination of overweight and obesity

The results for the anthropometric measurements from which overweight and obesity indicators were derived are shown in Table 2. Male nurses were evidently more likely to be taller on average than their female counterparts. However, in contrast, females were on average more likely to be heavier and for that matter have higher BMI than males. With regards to waist and hip circumferences, female nurses

Table 2: Anthropometric Measurements of Respondents with their various Indices classified by gender

Variables	Male	Female	P Values
Height (cm)	169.8±1.0	161.2±0.5	0.026
Weight (kg)	66.4±1.0	68.3±1.2	0.003
BMI (kg/m ²)	23.0±1.0	26.0±1.0	<0.001
WC (cm)	80.0±0.4	86.4±0.4	<0.001
HC (cm)	94.5±0.3	104.4±0.4	0.001
WHR	0.94±0.1	0.87±0.1	0.005

BMI – Body mass index; WHR – Waist-to-hip ratio; WC— Waist circumference; HC—Hip circumference

had on average bigger waist and hip lines than the males but still had lower mean WHRs than their male counterparts.

Using the WHO cut-offs as reference, the BMI categorizations for the study population (Figure 1) showed 2.7% of the nurses (0.9% for males and 1.8% for females) were underweight, 26.4%(8.2% males and 18.2% females) were overweight and 16.9%(1.4% males and 15.5% females) were obese. There was a significant association between gender and BMI (p<0.001). Thus, female nurses had a much greater tendency to be overweight and obese than their male counterparts.

With respect to central obesity, 54 (24.5%) were considered at risk based on their WHRs. Among these, 12 (5.5%) were males while the remaining 42 (19.0%) were females (Table 3). Clearly, female nurses were significantly more likely to be at risk for central obesity than their male counterparts (p<0.001). BMI and WHR were compared to show which of the two indicators was more sensitive at determining obesity. The outcome showed that 6 (2.7%) males and 10 (4.5%) females with normal BMI were at risk of central obesity. The implication is that BMI alone is not able to determine the degree of obesity/overweight in the study population especially when body fat distribution is considered.

Risk factors for overweight and obesity in the study population

Socio-demographics: Gender, marital status, age and number of years in service

The relationship between gender and overweight/

Table 3: Waist-to-Hip Ratio categorization of the Nurses with respect to gender

Variables	Male	Female	Total
Normal	62(83.8%)	104(71.2%)	166(75.5%)
At Risk	12(16.2%)	42(28.8%)	54(24.5%)
Total	74(100.0%)	146(100.0%)	220(100.0%)

Normal (<0.80 for females and <0.90 for males), At risk (>80 for women and >90 for men)

obesity has clearly been depicted with both BMI and WHR categories showing significant relationships with gender. It can therefore be inferred that female nurses are more likely to be overweight and obese as well as at risk for central obesity than their male counterparts.

The association between marital status and BMI was shown to be positive but of borderline significance ($p=0.048$). From the results, 10.0% of the unmarried nurses were overweight and 4.0% were obese with 15.0% of the married nurses being overweight while 12.7% were obese. With divorced and widowed nurses the percentages for overweight (0.5% and 0.9%) and obese (0.9% and 1.4%) were much lower. This shows that, married nurses were more likely to be overweight and obese than single nurses (never married, divorced and widowed). A significant positive correlation was obtained between age and BMI ($r=0.437$, $p=0.007$). The implication here is that as the nurses' age, their likelihood of becoming overweight or obese also increases.

To assess the influence of the number of years in service on BMI, a linear regression analysis (Figure 2) depicted a significant association between the number of years in service and their BMI ($p=0.047$). The BMI of the nurses is more likely to increase with number of years in service increases.

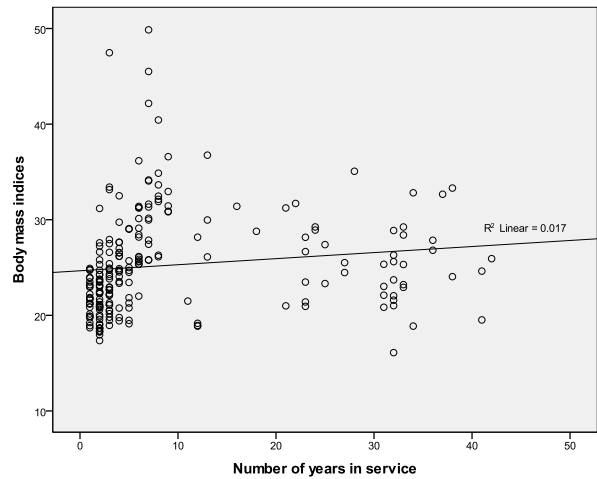


Figure 2: Scatter diagram showing Number of Years in Service with respect to BMI

Family Line of Fatness

Respondents were asked if any member of their family were perceived to be fat (overweight or obese). With respect to those who responded yes, 14.5% were overweight while 11.4% were obese. On the other hand, 11.8% of those who said no were overweight while 5.5% were obese. Those who had families perceived to be fat were significantly more likely to be obese and overweight ($p=0.042$) than those with no family relations perceived to be fat or obese.

Physical Activity: Means of transportation, exercise levels and television viewing time

With regards to the predominant means of trans-

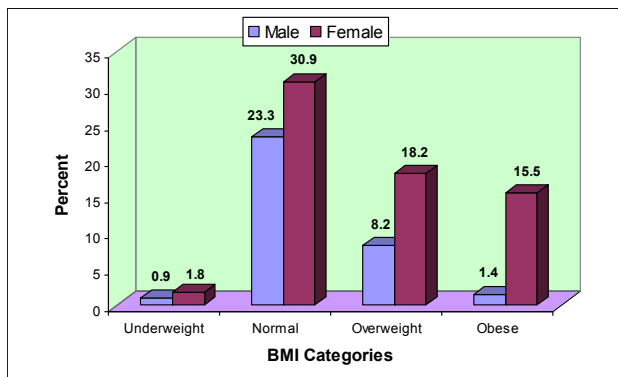


Figure 1: BMI Classification of Nurses with respect to Gender using WHO cut offs as reference. Underweight ($<18.5 \text{ kg/m}^2$), normal ($18.5 - 24.99 \text{ kg/m}^2$), overweight ($25-29.99 \text{ kg/m}^2$) and obese ($\geq 30 \text{ kg/m}^2$)

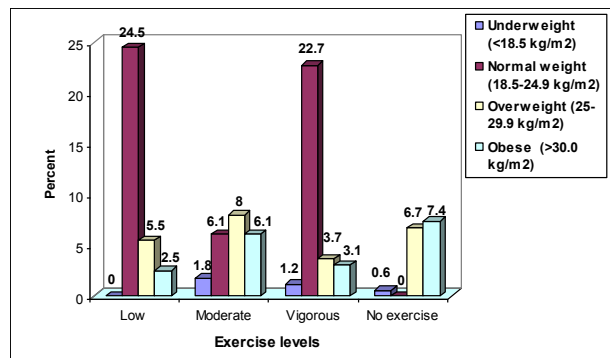


Figure 3: Association between Exercise levels and BMI categories

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portation to work by the respondents, about 50.9% went to work mainly by motorbike, 30.5% by car, 17.3% on foot and 1.4% by bicycle. Means of transportation was grouped as motorized (cars/motorbike) or un-motorized (foot/bicycle) to reflect the level of energy expended when going to work. However, there was no significant association between the means of transportation and BMI ($p=0.118$).

With respect to exercise levels reported by the respondents were grouped as vigorous, moderate, low and no exercise depending on the kind of activity involved (Figure 3). The association between exercise levels and BMI categories was positive and significant ($p=0.011$) and nurses who reported to indulge in no exercise regimen were more likely to be overweight (6.7%) and obese (7.4%) compared to those who reported to engaging in some form of exercise. The average time spent watching television daily was grouped into 1-3 hours, 4-6 hours and 7-9 hours. These groupings were compared to the BMI's (Figure 4). The relationship although positive was not significant ($p=0.087$).

Dietary Habits: Meal skipping and dietary diversity

With respect to meal skipping, 118 (53.6%) responded yes while 102 (46.4%) said no. Figure 5, presents the relation between meal skipping and BMI categories. The Chi-square test showed a positive significant association between meal skipping and BMI

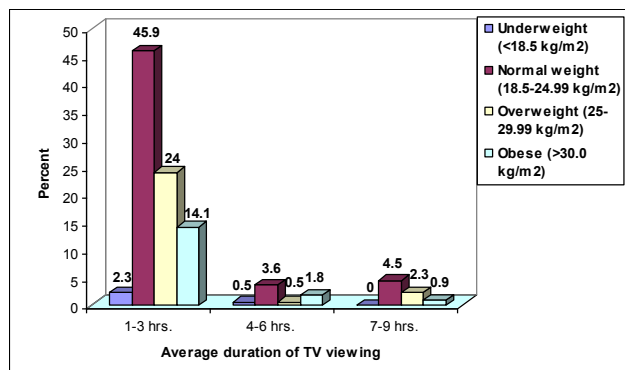


Figure 4: Association between times spent viewing television by the Nurses and BMI

($p=0.002$). Meal skipping was ascribed by some nurses to the nature of their work, which puts them on red alert, especially when they are on call.

The individual dietary diversity score (IDDS) was derived as a measure of diversity in the diet of respondents. Frequency of consumption of 8 different food groups were scored for dietary diversity and are as summarized in table 4. Correlation between the IDDS and BMI was negative and significant ($r=-0.246$, $p=0.001$) which meant that, obesity and overweight decreased as IDDS increased. Thus, nurses with less diverse dietary habits are more likely to be overweight and obese.

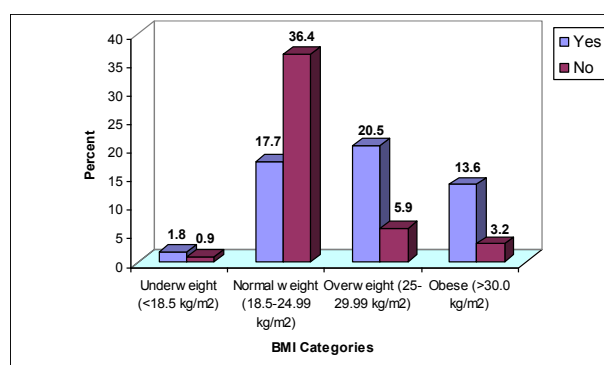


Figure 5: Relationship between Skipping Meals and BMI of the Nurses

Table 4: Dietary diversity scores derived from consumption of various food groups

Food group	Minimum	Maximum	Mean	SEM
Cereal and tubers	4	7	5.2	0.1
Meat and fish	1	7	5.4	0.1
Milk and milk products	0	3	2.1	0.1
Vegetables	0	4	1.2	0.1
Fruits	0	3	0.5	0.0
Pulses	0	3	0.5	0.0
Sugars	0	7	3.3	0.1
Fats and oils	1	7	5	0.1

DISCUSSION

Prevalence of overweight and obesity

This study has shown that underweight exists despite the high levels of overweight/obesity in the study population, which may lend credence to the existence and persistence of the phenomenon described as a ‘double burden’ of disease, which is common in many developing countries (Popkin, 2004, Prentice, 2006). The comparatively higher prevalence of overweight and obesity in this study population to that of the general Ghanaian population, according to the GDHS, (2008) (26.4% and 16.9% cf. 21% and 9%), may be explained by the a possibly higher socio-economic status in the study population compared to the average Ghanaian population or perhaps because the study population is largely female dominated, as these attributes have been known to be associated with overweight and obesity (Sobal and Stunkard, 1989; Benkeser *et al.*, 2012). The study findings are also consistent with those of Ogunjimi *et al.*, (2010) and Miller *et al.*, (2008) who reported prevalences of overweight/obesity of public health significance among nurses in Nigeria and the USA respectively. Nevertheless, the levels in the 2 studies cited were much higher perhaps because the USA study, for instance, reflected the very high levels in the American population and for that matter the female population, whilst in the Nigerian study this could be attributed to the use of female subjects only for the study.

The use of WHR for central obesity has also demonstrated that female nurses in this study are at a higher risk for chronic diseases such as hypertension, diabetes and arteriosclerosis than their male counterparts. This finding agrees with that of Azadbakht *et al.*, (2004) and Azadbakht *et al.*, (2005) who reported high prevalence of central obesity in Iranian women compared to men and also depicted their risk for such chronic diseases. The pattern of body fat distribution as measured by WHR has been reported to be a more important determinant of chronic diseases than general obesity (Wei *et al.*, 1997; Esmailzadeh *et al.*, 2006). Kissebah and Krakower, (1994) also established that WHR provides useful indices of abdominal fat accumulation and provides a better correlation with an increased risk of ill health than BMI

alone. This point is further echoed by the WHO statement that BMI can be used to estimate the prevalence of obesity within a population but cannot account for the wide variation in body fat distribution (WHO, 2005). The essence of the finding in this study of overweight and obesity levels that can be compared to those of affluent countries, coupled with the rates at which such countries are experiencing the fall outs from this epidemic, in terms of rising NCDs, may buttress the important point of Ogunjimi *et al.*, (2010) that if corrective measures are not put in place our care providers sooner than later would become care receivers.

Risk factors for overweight and obesity in the study population

Clearly, socio-demographic and socio-economic status are related to overweight and obesity in many populations (Sobal and Stunkard, 1989; Benkeser *et al.*, 2012), thus the finding in this study of significant associations between gender, marital status as well as age and overweight or obesity only goes to buttress this point. First, female gender is a significant risk factor for overweight and obesity in this study which conforms to the general trend for gender relationships with BMI in several other studies. For instance, studies from developing countries including Ghana have shown that obesity is more common in women than men (Martorell *et al.*, 2000; Amoah, 2003a; Azadbakht *et al.*, 2005). This gender trend also occurs in industrialized countries (Flegal, 2002; Odom, 2006) where fat intake as well as genetic predisposition is supposed to contribute to it (Heitmann *et al.*, 1995). The reasons for this association of overweight/obesity with gender may be explained from West African or Ghanaian cultural and historical perspectives.

A social desirability for overweight and obese women in West Africa is often cited together with historical records showing that some ethnic groups in Africa preferred overweight women (Jackson *et al.*, 2005; Agyemang *et al.*, 2008; Fezeu *et al.*, 2008) whilst some embraced cultural practices that encouraged female obesity (as in the pre-marital “fattening rooms” of Nigeria) (Brink, 1995; Benkeser *et al.*, 2012).The perception of overweight

and obesity as a sign of good health, wealth and beauty in many African countries (Amoah, 2003b) may also fuel this growing epidemic of obesity in females especially.

Secondly, married nurses in this study were more likely to be overweight and obese than single nurses (never married, divorced and widowed) which may imply that marital status of nurses was likely to have an influence on their BMI. This finding conforms to that of Mohsen, (2008) and Lipowk-z *et al.*, (1998), who reported in their respective studies that married persons, were more likely to be overweight and obese than never married individuals. The finding is also supported by that of Ogunjimi and his colleagues (2010) who found a high degree of obesity in married than unmarried female nurses in Nigeria. Marriage may come with much physical, psycho-social and financial support from partner and relations, which may facilitate comfort and a tendency for decreased physical activity or energy expenditure together with other lifestyle changes that may be associated with weight gain. For most marriages also fecundity or number of children is expected to increase, which culturally comes with lessening of physical activity and increased food intake on the part of the female spouse. Benkeser *et al.*, (2012) in their study in women in Accra established that given birth to 2 or more children was a higher risk for obesity and being unmarried and living in a rural environment rather had a protective effect.

Thirdly, age has been established as a very important predictor of overweight/obesity among both men and women (Lipowk-z *et al.*, 1998) as our study findings confirm. In Ghana Amoah (2003b) also observed that obesity increases with age. The propensity for obesity in both men and women as they age may be explained by pubertal and body fat compositional changes associated with the hormonal influences that come with sexual growth and development. The female hormones are more effective in this respect as reproduction and other physiological functions are at stake.

The ageing of nurses may correspond with their length of service, which may mean that longer serv-

ing nurses would have a greater tendency to be overweight and obese as the findings suggest. A plausible explanation to this finding comes from the consideration that nurses' may tend to acquire an increased sedentary routine and expend less energy as they assume senior or supervisory positions in the service. This can increase the risk for becoming overweight and obese. Notwithstanding the socio-demographic relationships with overweight and obesity depicted in this study, the contribution of heredity cannot be overruled as demonstrated by the greater tendency for individuals with a family member perceived as overweight or obese to be overweight or obese themselves. A study by Senekal *et al* in 2003 also showed that an individual is likely to become overweight having at least one overweight family member, more specifically a parent.

Many studies have shown that unhealthy lifestyle behaviors, particularly lack of exercise which invariably leads to low physical activity contribute significantly to overweight and obese tendencies (Steyn *et al.*, 2006; Kruger *et al.*, 2005; Senekal *et al.*, 2003; Schmitz *et al.*, 2000). In this respect, our findings which showed a significant relationship between reported exercise levels and BMI lends support. In addition, Television (TV) viewing for longer periods connoting a sedentary lifestyle has been associated with increasing obesity tendencies (Gortmaker *et al.*, 1996; Crespo *et al.*, 2001; Hu *et al.*, 2003). Even though TV viewing related obese tendencies occurred mostly in children (Gortmaker *et al.*, 1996; Crespo *et al.*, 2001) who were more likely to indulge in an increased energy intake concurrently, the study by Hu *et al.*, (2003) revealed that women who watched much TV were more likely to be predisposed to obesity and diabetes mellitus type II. In our study, it is likely that watching of TV for more than 3 hours was occasioned by intermittent and scheduled viewing of programmes perhaps to the extent that some of the time was spent doing other activities whilst the TV was switched on, and therefore the true picture of the relationship was not revealed. Furthermore, the preponderant use of motorized means of transport to work and other places though common among the nurses did not

yield any significant relationship with BMI simply because many of the nurses also footed or used bicycles as alternative means.

A particular habit of meal skipping is becoming a norm rather than an exception with most professionals as they become inundated with work. Ironically, the meal that is skipped most is breakfast, which happens to be the most important meal of the day. With the nursing profession, unexpected or emergency cases present at any time with some taking quite a long time to resolve, which more often than not obligates meal skipping. The significant finding in our study which relates meal skipping to overweight and obesity shows that meal skipping can be a contributing factor to weight gain and obesity as established in the studies by Jenkins *et al.*, (1994), Fabry, (1964) and Yunsheng *et al.*, (2003) who reported that skipping meals was associated with a significantly higher risk for overweight and obesity.

Obviously, meal skipping, especially breakfast, is an inappropriate dietary habit with nutritional consequences (Rashidi *et al.*, 2007). The evidence for skipping meals such as breakfast does not only interrelate with obesity but also leads to an increased vulnerability to undernutrition due to restricted food intake (Niclas *et al.*, 1998). Thus, skipping of meals possibly engenders a poorly diversified diet, which may come as part of the nutrition transition associated with the pressures of urbanization and increased workload. In the work of Sarrafzadegan *et al.*, (2009) an inverse association between IDDS, obesity and abdominal adiposity was observed among female students of Isfahan University in Iran, lending support to the inter-relationship between less than optimal dietary intakes and obesity.

CONCLUSION

Results from this study have revealed that prevalence of overweight and obesity is high among the nurses within the Tamale metropolis mediated heavily by socio-demographic characteristics such as age, gender and marital. A predominance of physical inactivity and dietary habit of meal skipping as well

as low dietary diversity were found to be significant contributors to overweight and obesity among the nurses.

By virtue of their occupation and various sedentary tendencies associated with it, the nurses are at significant risk of becoming overweight and obese as well as developing central obesity, which may have serious implications on their health and for that matter their productivity.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Influence of physical restraint on the onset of experimentally induced diabetes mellitus

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The role of intermittent repeated physical restraint on the onset of diabetes mellitus (DM) was investigated in this study. The study compared the onset of DM in mice dosed with streptozotocin (STZ), a DM-inducing drug, with immediate subsequent exposure to either physical restraint stress or non-exposure to the stress. Sixty mice were randomly assigned to 6 equal groups: 0 mg kg⁻¹ STZ with no stress, 0 mg kg⁻¹ STZ with stress, 25 mg kg⁻¹ STZ with no stress, 25 mg kg⁻¹ STZ with stress, 50 mg kg⁻¹ STZ with no stress, 50 mg kg⁻¹ STZ with stress. Blood glucose, body weight and food consumption were regularly determined during the study. On day 18, mice were killed and blood for corticosterone determination was collected. Increase in STZ dosage or physical restraint stress lowered bodyweight on days 4-18 ($P < 0.05$). Increasing STZ dosage elevated the blood glucose on day 7-18 ($P < 0.05$). Restraint lowered blood glucose on day 11-18 ($P < 0.05$). Interaction between both factors was significant on day 11-18 ($P < 0.05$). Nine out of 10 of the 50 mg kg⁻¹ STZ no-stress mice and 2 out of 10 of the 50 mg kg⁻¹ STZ stress mice developed DM. Physical restraint was a more important predictor for whether a mouse would have been diabetic or not. Physical restraint delayed the onset of diabetes mellitus.

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Keywords: Diabetes mellitus; physical exertion; restraint stress; streptozotocin

INTRODUCTION

Diabetes is a huge global health issue and is expected to continue growing. Currently, almost 10% of the world's adult population has diabetes (WHO, 2013). Diabetes mellitus has been defined as a metabolic disorder of multiple origins characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both (Alberti and Zimmet, 1998). The well-known classifications of diabetes, type 1 and type 2 (Castano and Eisenbarth, 1990, Alberti and Zimmet, 1998) ensure patients are classified based on pathogenesis rather than treatment. Diabetes type 1 is a result of the destruction of the beta cells of the pancreatic islets whereas diabetes type 2 is characterized by the mal-

function of insulin action or secretion, either of which may be more prevalent, but both are usually seen at the time of diagnosis (Castano and Eisenbarth, 1990, Roep, 2008). In general, the development of diabetes involves several disease-causing processes, including processes which destroy the beta cells of the pancreatic islets, causing insulin deficiency, and others that cause resistance to the function of insulin (Alberti and Zimmet, 1998). However, much remains to be learned about diabetes' pathogenesis and relationships with environmental factors, such as physical exertion and other forms of stress.

Stress is the body's non-specific response to real or perceived threats/demands (Selye, 1976). It may be categorized into two types: acute and chronic. During acute stress the hormones cortisol and adrenaline are released, causing increased blood pressure and heart rate and heightened immune system and memory, which can be helpful for a short period of

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time. The response to chronic stress, however, can be detrimental. Blood pressure, heart rate, appetite, bodyweight, cholesterol, triglyceride, and blood sugar levels have been shown to change during chronic stress. These are not only risk factors for heart disease, atherosclerosis, stroke and obesity, but also diabetes (McEwen, 2008). For example psychological stress including physical restraint stress has been shown to enhance the production of immunosuppressive cytokines linked to an increased occurrence of infectious disease, which demonstrates that the immunosuppressive actions of stress do in fact translate into significant adverse health effects (Curtin *et al.*, 2009).

Cortisol levels are known to rise in response to stress. Because the main effect of cortisol involves raising blood glucose levels, we therefore hypothesized, in the present study, that stress including physical restraint stress (that normally involves physical exertion) would accelerate the onset of chemically-induced diabetes mellitus in otherwise normal healthy Swiss ICR mice.

MATERIALS AND METHODS

Experimental procedure

Sixty hsd:ICR(CD-1®) mice were randomly assigned to 6 groups of 10 mice each. Two factors, physical restraint stress at 2 levels and streptozotocin at 3 levels (dosages were in mg kg⁻¹ bodyweight), were arranged in a factorial manner to produce 6 treatments: 0 mg kg⁻¹ STZ with no stress (0 mg kg⁻¹ STZ no-stress), 0 mg kg⁻¹ STZ with stress (0 mg kg⁻¹ STZ stress), 25 mg kg⁻¹ STZ with no stress (25 mg kg⁻¹ STZ no-stress), 25 mg kg⁻¹ STZ with stress (25 mg kg⁻¹ STZ stress), 50 mg kg⁻¹ STZ with no stress (50 mg kg⁻¹ STZ no-stress), 50 mg kg⁻¹ STZ with stress (50 mg kg⁻¹ STZ stress). Mice were housed individually and allowed 3 days to acclimatize before experimentation.

Mice received once-a-day intraperitoneal injections of the assigned dose of a diabetes-inducing chemical, streptozotocin (STZ) in citrate buffer or citrate buffer alone for the first three days of the study. Streptozotocin-treated mice are commonly used to model diabetes type 1 due to the ability of strepto-

zotocin (STZ) to destroy the insulin-producing beta cells of the pancreatic islets of Langerhans (Reagan *et al.*, 1999, Maiese *et al.*, 2007). Baseline animal weight, feed weight, and glucose levels were taken on day 0 for all mice and STZ or buffer injections were given.

Non-fasting glucose measurements were taken three times a week beginning on day 0 for both groups using blood glucose meters (Freestyle Freedom Lite, Catalog number 70914, NDC 99073-0709-14, Distributed by Abbott Diabetes Care Inc) and appropriate test strips (Freestyle Lite Blood Glucose Test Strips). Mice were weighed twice each week. Feed weight was taken two times per week. Feed was refilled and reweighed as needed to calculate food consumption. Mice exposed to physical restraint stress as described by Bonneau *et al.*, (1993) were placed in 50 ml well-ventilated tubes packed lightly with approved nesting material for 6 hours beginning at approximately the same time each day during which time the control mice were free in their cages but without access to feed and water. After the 6 hour-period the stressed mice were returned to their cages and both the stressed and the control mice were again given access to feed and water. The study lasted for 18 days. Such exposure to physical restraint stress for 6 hours per day for 18 days was considered chronic stress. This was in line with the study of Gao *et al.*, (2006) who considered physical restraint stress for 6 hours per day for 21 days to be chronic stress while one time 6-hour restraint was considered acute stress. At the end of the 18 days of the study, mice were euthanized and blood was collected for serum preparation. The animal protocol was approved by the University Committee on Animal Care (UCAC) at East Tennessee State University, Johnson City, TN, a AALAC accredited research institution.

Corticosterone Assay

The corticosterone assay was performed according to a colorimetric competitive enzyme immunoassay method outlined by the manufacturer of a commercial kit (Assay Designs® Corticosterone Enzyme Immunoassay Kit, Ann Arbor, MI, Catalog No. 900-097). Standards, blanks, samples and reagents were

placed in the designated wells as per the kit's instructions. The plates were then covered, incubated at room temperature and thereafter contents were decanted. The wells were then washed and conjugate solution added to the total activity wells before stopping the reaction in all wells. The extent of colorimetric change in each well was read using a microplate reader (Benchmark Microplate Reader, Bio-Rad, Hercules, CA) at a wavelength of 405 nm with a correction between 570 and 590 nm.

Statistical Analysis

The SAS statistical software (version 9.2, SAS Institute Inc. 2002, Cary, NC) was used to perform statistical analyses (SAS, 2002). Since there were two factors: STZ and stress, a two-way ANOVA (analysis of variance) was used. The study was a factorial one in which the response is observed at all factor-level combinations of the independent variables. Two-way ANOVA model splits the total variability into four sources of variability, which in this case are: the main effects of STZ, the main effects of stress, the possible interaction between STZ and stress, and the unexplained variability from all sources not accounted for by the main effects and interaction, known as error (Ott *et al.*, 2001). In order to compare changes among individuals, baseline values were taken. Baseline measurements were also used to ensure that the independence assumption of ANOVA model was not violated by checking for randomization of the measurements (DeVeaux *et al.*, 2008). ANCOVA (analysis of covariance) was used to account for the effect of baseline measurement values when analyzing the values for any one subsequent specific time (Wildt and Ahtola, 1978). Baseline values were used as covariates when studying the difference in values between treatments in subsequent times. In this manner, one could determine if the baseline level was an important factor.

Data sets of the repeated measures nature (with multiple measurements of a response variable on the same experimental unit) were analyzed using repeated measures. Repeated measures analysis was performed on bodyweight, glucose and food consumption and the Akaike information criterion (AIC) was used to select the method to be used for correlation.

Repeated measures analysis was done to investigate whether there were significant differences within groups at the different times when the data were collected and also between the different treatment groups. Longitudinal analysis (Fitzmaurice *et al.*, 2008) was applied to blood glucose, body weight and cumulative food consumption data and response profiles for the measurements taken at different times were examined to characterize the patterns of change in the respective response variable over time in the different groups.

The two factors (STZ and physical restraint stress) were evaluated using logistic regression procedure in SAS for their suitability in predicting diabetes development (persistent blood glucose level of at least 200 mg dl⁻¹, (Padmanabhan *et al.*, 2006) in the mice. Differences were considered significant at $P < 0.05$.

RESULTS

In general, statistical analyses revealed that STZ, physical restraint stress, and their interaction became statistically significant in causing the measurable differences in the various response (dependent) variables among treatment groups. When repeated measures analysis was performed on bodyweight, glucose and food consumption, the interaction between stress and STZ was dependent on the day of the measurement (Tables 1, 2 and 3).

Blood Glucose

Persistent non-fasting blood glucose level of at least 200 mg dl⁻¹ was considered as evidence of the mice having developed diabetes mellitus in this study (Padmanabhan *et al.*, 2006). There were no significant differences between the baseline blood glucose values among the different treatment groups ($P > 0.05$, Table 1). However by day 7 of the study, the differences, using ANOVA, among mice dosed with different levels of STZ became significant ($P < 0.013$), although there was no difference as yet between stressed and non-stressed mice (Table 1). STZ becoming significant meant that different groups of mice no longer had the same glucose levels. The 50 mg kg⁻¹ STZ mice showed significantly higher blood glucose level than the lower

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STZ doses (0 and 25 mg kg⁻¹). STZ was also significant on day 7 using ANCOVA with glucose day 0 as a covariate. The difference was clearer ($P<0.0052$) when using ANCOVA with glucose day 0 as a covariate because baseline variability was negated. The effect of STZ on blood glucose level remained significant for the rest of the experimental days with the higher STZ dose mice showing significantly higher blood glucose compared to the lower STZ dose mice (Table 1). The mice that received 0 or 25 mg kg⁻¹ STZ showed no difference in mean blood glucose between the stressed and non-stressed mice (Table 1).

The effect of physical restraint stress on blood glucose became significant from day 11 and then on day 16 until the end of the experiment on day 18. Physical restraint stress on these days lowered the blood glucose levels (Table 1). By day 9 of the experimental period, there was a mild interaction between the ef-

fect of STZ and physical restraint stress on blood glucose level ($P<0.0609$, Table 1). An interaction occurs when the effects of one factor change for the different levels of another factor. By day 11, the STZ-stress interaction was significant ($P<0.0029$) and remained significant ($P<0.05$) for the rest of the experimental period. Interaction between STZ and physical restraint stress was noted at the highest STZ dose (50 mg kg⁻¹) group and it was this group that had a much higher mean for glucose change from the baseline values. STZ affected the blood glucose of mice differently depending on whether they were stressed or not. Surprisingly it was the stressed mice that showed lower levels of blood glucose compared to the non-stressed mice (Table 1). Nine out of 10 of the 50 mg kg⁻¹ STZ no-stress mice and 2 out of 10 of the 50 mg kg⁻¹ STZ stress mice developed diabetes mellitus (persistent blood glucose of ≥ 200 mg dl⁻¹). Hyperglycemia in these mice was observed as early as day 4 and con-

Table 1: Blood glucose level (means, mgdl⁻¹) in mice dosed different levels of STZ and then subsequently either stressed or not stressed for 18 days (n=10)

STZ	Stress	Day 0	Day 2	Day 4	Day 7	Day 9	Day 11	Day 14	Day 16	Day 18
<i>Simple effect means</i>										
0	No	126	100	142	121	121	114	97	111	118
	Yes	121	107	143	126	126	121	122	112	138
25	No	128	123	132	132	120	135	118	114	139
	Yes	132	111	118	117	120	124	120	127	118
50	No	123	105	163	168	210	262	265	288	346
	Yes	120	112	135	146	148	162	154	183	191
<i>Main effect means</i>										
0		122	104	143	123	124	118	110	111	128
25		130	117	125	125	115	129	119	120	128
50		122	109	149	157	179	212	210	236	269
	No	125	110	146	140	147	170	160	171	201
	Yes	124	110	132	130	131	136	132	141	149
<i>Source of Variation</i>		<i>Probability</i>								
STZ		0.3626	0.2769	0.2179	0.0052	0.0006	0.0001	0.0001	0.0001	0.0001
Stress		0.9445	0.9098	0.2309	0.2549	0.2575	0.0097	0.0954	0.0419	0.0014
STZ * Stress		0.8476	0.4119	0.5739	0.4765	0.0609	0.0029	0.0026	0.003	0.0001
SD ¹		22	26	43	35	53	50	63	57	60

¹SD = Pooled standard deviation

tinued for the rest of the study period.

Unstructured correlation was used for the repeated measures analysis because it yielded a lower value for the Akaike information criterion (AIC). The effects of STZ, stress, day and interactions STZ-stress, STZ-day, stress-day and STZ-stress-day on blood glucose level were all significant. Similar to the ANOVA results, the repeated measures model showed that STZ, physical restraint stress, day and their interactions were significant factors in causing differences in blood glucose among the six treatment groups. Group response profiles for mean blood glucose levels showed that while the mean glucose levels for the 0 and 25 mg kg⁻¹ STZ stress and no-stress groups remained relatively stable, it was evident that the blood glucose levels of the 50 mg kg⁻¹ STZ no-stress group increased throughout the study (Figure 1) whilst the glucose levels of the 50 mg kg⁻¹ STZ stress group increased only slightly. Profiles for all other groups except the 50 mg kg⁻¹ STZ no-stress seemed to be parallel to each other implying that blood glucose levels in these groups changed in the

same manner across time, regardless of the treatment group.

When the two factors (STZ and physical restraint stress) were evaluated for their suitability in predicting diabetes development in the mice, restraint was significant ($P < 0.0130$). The predicted probabilities were 98.1% concordant. This means that the model was 98.1% correct in its prediction of all possible concordant pairs of mice that developed diabetes with those that did not develop diabetes.

Bodyweight

Baseline bodyweight values were not significantly different among all the treatment groups ($P > 0.005$), a confirmation of the randomness of the assignment of the mice to the 6 treatment groups (Table 2). By day 4 of the experiment, the effects of STZ and physical restraint stress were significantly affecting bodyweight ($P < 0.05$, Table 2). This remained so for the rest of the experimentation period. Higher levels of both factors significantly reduced the body weight of the mice. The highest

Table 2: Bodyweight (means, g) in mice dosed different levels of STZ and then subsequently either stressed or not stressed for 18 days (n=10).

STZ	Stress	Day 0	Day 4	Day 9	Day 11	Day 15	Day 18
<i>Simple effect means</i>							
0	No	32	33	34	34	34	36
	Yes	32	31	31	31	32	33
25	No	32	33	34	34	34	35
	Yes	32	31	31	31	32	33
50	No	32	31	32	32	32	33
	Yes	32	30	30	30	31	32
<i>Main effect means</i>							
0		32	32	32	33	33	34
25		32	32	32	33	33	34
50		32	31	31	31	31	32
	No	32	32	33	33	33	35
	Yes	32	31	31	31	32	32
<i>Source of Variation</i>		<i>Probability</i>					
STZ		0.5582	0.0148	0.0386	0.0253	0.0093	0.0121
Stress		0.587	0.0003	0.0001	0.0001	0.0003	0.0001
STZ * Stress		0.7308	0.8681	0.6315	0.703	0.5603	0.5412
SD ¹		1.6	1.5	1.8	1.8	1.9	1.9

¹ SD = Pooled standard deviation

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STZ dosed animals weighed significantly less ($P<0.05$) compared to either the 0 or 25 mg kg⁻¹ STZ dosed animals. Also the mice exposed to physical restraint stress weighed significantly less ($P<0.05$) compared to non-stressed animals. There were no significant interaction effects of STZ and physical restraint stress on bodyweight.

Unstructured correlation was used for the repeated measures analysis because it yielded a lower value for the Akaike information criterion (AIC). Repeated measures analysis of body weight data showed that STZ, physical restraint stress, day and stress-day interaction had significant effects on bodyweight. Like the ANOVA results, the repeated measures analysis showed that STZ and physical restraint stress were significant factors in causing the differences in body weight among the six treatment groups. Group response profiles for the mean body

weights showed that all treatment groups lost weight in the beginning before starting to gain weight with exceptions in the 0 and 25 mg kg⁻¹ STZ no-stress groups (Figure 2). The 50 mg kg⁻¹ STZ no-stress group lost the most weight and remained the lightest group in the study. There was very little variability existing among the treatment groups on day 0 compared with day 18. The higher dose of STZ caused a decrease in bodyweight as did stress.

Food consumption

There were no significant effects of STZ or physical restraint stress on cumulative food consumption (Table 3). Repeated measures analysis on food consumption however showed that the effect of day on cumulative food consumption was significant ($P<0.05$). None of the other factors had a significant effect on food consumption. Differences from day-to-day were however expected because meas-

Table 3: Cumulative food consumption (mean, g) in mice dosed different levels of STZ and then subsequently either stressed or not stressed for 18 days (n=10).

STZ, mg kg ⁻¹	Stress	Day 0	Day 8	Day11	Day15	Day18
<i>Simple effect means</i>						
0	No	0	60	87	116	139
	Yes	0	51	73	99	118
25	No	0	54	71	96	113
	Yes	0	51	69	95	112
50	No	0	67	91	121	142
	Yes	0	51	69	94	112
<i>Main effect means</i>						
0		0	56	80	108	128
25		0	52	70	95	112
50		0	59	80	107	127
	No	0	60	83	111	131
	Yes	0	51	70	96	114
<i>Source of Variation</i>		<i>Probability</i>				
STZ			0.5914	0.4956	0.4619	0.4222
Stress			0.0682	0.0935	0.1145	0.1254
STZ * Stress			0.5651	0.5773	0.5188	0.5421
SD ¹			20	30	35	42

¹ SD = Pooled standard deviation

urements were cumulative in nature. The group response profiles for cumulative feed consumption showed that the profiles remained parallel to each other with more variability among groups being observed on day 18 than at the beginning, thus giving an indication that the feed consumption increased similarly for all groups.

Corticosterone

The two-way ANOVA analysis on serum corticosterone levels at the end of the study indicated that STZ and physical restraint stress and their interaction had significant effects on corticosterone levels (with P values of <0.0048 , <0.0001 , and 0.0010 , respectively). Simple effect means of serum corticosterone levels (pg ml^{-1}) for 0 mg kg^{-1} STZ no-stress, 0 mg kg^{-1} STZ stress, 25 mg kg^{-1} STZ no-stress, 25 mg kg^{-1} STZ stress, 50 mg kg^{-1} STZ no-stress, 50 mg kg^{-1} STZ stress were 1375, 1278, 400, 3064, 884, 6164, respectively. Main effect means of serum corticosterone levels (pg ml^{-1}) for the different levels of STZ (0 , 25 and 50 mg kg^{-1} bodyweight) were 1327, 1732 and 3524, respectively and those of no-stress and stress treatments were 886 and 3502, respectively. Increasing the STZ levels had an effect of raising the serum corticosterone level with stressed mice showing higher serum corticosterone levels.

DISCUSSION

The drug streptozotocin (STZ) used in this study destroys the beta cells of the pancreatic islets and it

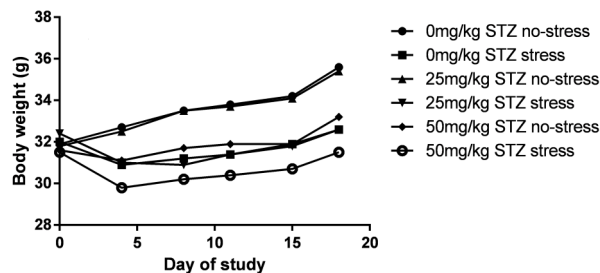


Figure 1: Response profile for blood glucose level (means, mg dl^{-1}) in mice dosed different levels of STZ (mg kg^{-1} bodyweight) and then subsequently either stressed or not stressed for 18 days ($n=10$).

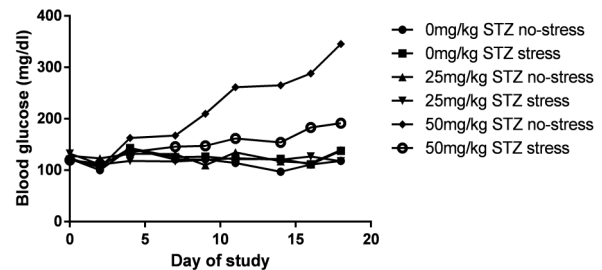


Figure 2: Response profile for bodyweight (means, g) in mice dosed different levels of STZ (mg kg^{-1} bodyweight) and then subsequently either stressed or not stressed for 18 days ($n=10$).

is commonly used to induce diabetes mellitus type 1 in study animals. Low doses of streptozotocin have been shown to produce diabetes mellitus. For example, a low-dose STZ regimen of 50 mg kg^{-1} injected intraperitoneally for 5 consecutive days in fasted mice produces hyperglycemia within 2 weeks (Breyer *et al.*, 2005).

In some studies it has been suggested that the incidence of diabetes may vary depending upon environmental factors such as stress (Fitzpatrick *et al.*, 1992). In the study by Fitzpatrick *et al.*, (1992), serum glucocorticoid concentrations in basal and stress conditions were measured in non-obese diabetic mice and C57BL/6 control mice. It was found that the diabetic mice generally exhibited a higher corticosterone response than the controls (Fitzpatrick *et al.*, 1992). In the present study, STZ was found to be the source of the observed difference in corticosterone levels among the different groups. These observations are in agreement with previous findings where STZ-induced diabetes was accompanied by elevated levels of serum corticosterone (Mizuno *et al.*, 1999, Oishi *et al.*, 2004). Another study that also observed high resting levels of plasma corticosterone in diabetic rats concluded that those observations suggested that diabetic rats were in a chronic stress condition (De Nicola *et al.*, 1977).

Interestingly, in this study the non-stressed 25 mg kg^{-1} and 50 mg kg^{-1} STZ mice had higher glucose

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levels but it was the stressed 25 mg kg⁻¹ and 50 mg kg⁻¹ STZ mice that had higher corticosterone levels. Many studies have researched the effects of stress on diabetic mice and rats (Huang *et al.*, 1981, Meehan *et al.*, 1987, Fitzpatrick *et al.*, 1992, Korolkiewicz *et al.*, 1999, Reagan *et al.*, 1999, Bates *et al.*, 2007, Bazhan *et al.*, 2007). Reagan *et al.*, (1999) examined the neurological changes induced by 7 days of physical restraint stress in STZ diabetic rats and found that the hippocampus of diabetic rats was extremely susceptible to stress. This research group reported that diabetic rats showed dendritic atrophy of pyramidal neurons, increased GLUT3 mRNA and protein expression in the hippocampus and stress additionally caused an increase of receptors for insulin-like growth factor (IGF) in the hippocampus (Reagan *et al.*, 1999).

In a study by Korolkiewicz *et al.*, (1999), using rats made diabetic by a single 70 mg kg⁻¹ STZ injection 5 weeks prior to the study showed that stressful stimuli such as food deprivation and cold challenge contributed to the elevated susceptibility of diabetic gastric mucosal damage. Bazhan and others (Bazhan *et al.*, 2007) found that repeated light emotional stress decreased the development of obesity and diabetes type 2 in mice with Agouti yellow mutation which produces an obese diabetic phenotype (Bazhan *et al.*, 2007). Using borderline, overt, or severe diabetic mice induced by STZ, Meehan *et al.*, (1987) studied glycemic responses of mice to the stress of a resident-intruder encounter and stress of blood drawing from the retro-orbital sinus. They found that plasma glucose elevation in overtly and severely diabetic mice is not as specific to behavior as in non-diabetic mice (Meehan *et al.*, 1987). Bates *et al.*, (2007) found that intermittent restraint delayed hyperglycemia and improved glucose control in Zucker diabetic fatty rats.

In many of the above studies stress was commonly applied to already diabetic animals while in the present study low levels of STZ were administered and physical restraint stress was immediately applied to mice even before exhibiting diabetic symptoms. Baseline values for glucose and body weight obtained in this study were not significantly different

from each other. This was an important foundation as it meant that there was no bias among treatment groups before treatments began. The effect of STZ on blood glucose levels became significant by day 7 of the experimental period. This observation is in agreement with the findings of Mizuno *et al.*, (1999) who noted that, one week after STZ injection, they could define mice as diabetic when they exhibited plasma glucose greater than 300 mg dl⁻¹.

Surprisingly, by day 9 and the subsequent days, the 50 mg kg⁻¹ STZ stressed mice exhibited lower levels of glucose than their non-stressed counterparts. This finding is contrary to expectations of the effects of stress on blood glucose. Stress is normally accompanied with a rise in glucocorticoids with a resultant rise in blood glucose, a condition that would be expected to favour the development of diabetes mellitus. There have been similar findings of reduced hyperglycemia in certain types of stress such as suspension by nape of neck stress (Kosovskii *et al.*, 1988), intermittent restraint (Bates *et al.*, 2007), and light repeated physical restraint stress (Bazhan *et al.*, 2007).

Research by Kosovskii *et al.*, (1988) comparing types of stress and the development of diabetic syndrome found that mice stressed through cavitory operation exhibited the signs of diabetes while those stressed through suspension by nape of neck did not. They suggested that the differences could be attributed to the fact that cavitory operation resulted in limited mobility while mice stressed by suspension had increased movement while trying to escape. Reduced hyperglycemia in mice stressed by suspension by nape of neck could provide one possible explanation for the reduced hyperglycemia in restraint-stressed mice in the present study: while the mice were being stressed by physically restraining them, they were working escape, which was a form of exercise and this seemed to have reduced the occurrence of high blood glucose levels in these mice. Further support for a role of physical restraint in reducing or delaying hyperglycemia is suggested by the hampering of development of diabetes type 2 by repeated physical restraint stress (Bazhan *et al.*, 2007) and also by the delayed hyper-

glycemia and improved glucose control by intermittent restraint (Bates *et al.*, 2007).

In the above studies (Kosovskii *et al.*, 1988, Bates *et al.*, 2007, Bazhan *et al.*, 2007) stress was applied to animals genetically predisposed to diabetes. In the present study, however, stress was applied to normal healthy Swiss ICR mice not genetically predisposed to diabetes. Even in these animals where diabetes mellitus could develop due to chemical exposure, the presence of physical restraint stress (that involves physical exertion of the animals) slowed down the development of consistent hyperglycemia associated with diabetes mellitus. The findings in the present study are supported by observations of Huang and others (Huang *et al.*, 1981) who stressed STZ-injected mice (60 mg kg⁻¹ body weight) through shock stimulation and found that none of the mice developed diabetes mellitus type 1 if they were stimulated an hour after STZ injection whereas non-stimulated mice developed hyperglycemia and became diabetic within 6 and 8 weeks after STZ injection. Indeed it would seem to suggest that stress that causes physical exertion may mitigate development of hyperglycemia.

From this study, STZ, stress and their interaction were significant factors in causing the differences in blood glucose levels among the six treatment groups. Group response profiles for glucose showed that the 50 mg kg⁻¹ STZ non-stress group had the greatest increase in glucose levels suggesting that physical restraint stress, in some way, protected the mice from development of hyperglycemia. Group response profiles for body weight showed that physical restraint stress seemed to have made mice in all groups to lose weight initially with the exception of 0 mg kg⁻¹ STZ and 25 mg kg⁻¹ STZ non-stress groups respectively. This finding is in agreement with that of Reagan and others (Reagan *et al.*, 1999) who observed that body weight significantly decreases as the diabetic state develops in STZ-injected mice. It was interesting to note that even though there were no significant differences in the cumulative amount of feed consumed, there were significant differences in bodyweight among the different groups. Because the higher bodyweight was in non-stressed mice, it may

be that physical restraint stress may have led to a higher energy consumption compared to the stressed animals thus resulting in body weight differences.

Serum corticosterone levels were affected by both STZ and stress levels with stressed mice showing higher corticosterone levels. Mice on higher STZ levels showed elevated serum corticosterone levels when they were stressed perhaps indicating that STZ may in some way be predisposing the mice to easier elevation of corticosterone when mice are stressed. Logistic regression analysis showed that physical restraint stress was a significant factor in the prediction of the development of diabetes (hyperglycemia of over 200 mg dl⁻¹). The physical struggle (exertion) of the mice during restraint may have mitigated the development of diabetes in stressed mice.

CONCLUSION

The present study suggests that restraint stress that tends to lead to increased physical exertion may attenuate the onset of diabetes mellitus type 1 in mice. Physical restraint was predictive for whether a mouse would be diabetic or not. It seemed to mitigate the development of diabetes mellitus (persistent hyperglycemia) in mice and hence delayed the onset of diabetes mellitus.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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