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L-glutamine ameliorates adipose-hepatic dysmetabolism in OC-treated female rats

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Abstract

Adipose dysfunction and inflammation with or without hepatic defects underlie metabolic obesity. Glutamine (GLU) improves glucoregulation and metabolic indices but its effects on adipose function and hepatic lipid deposition in estrogen-progestin oral contraceptive (EPOC) users are unknown. Therefore, we hypothesized that GLUT supplementation would protect against adipose dysfunction and excess hepatic lipid influx and deposition in EPOC-treated animals by suppressing adenosine deaminase/xanthine oxidase (ADA/XO) activity and improving glucose-6-phosphate dehydrogenase (G6PD)-dependent antioxidant defense. Female Wistar rats weighing 150–180 g were allotted into control, GLUT, EPOC and EPOC + GLUT groups (six rats/group). The groups received vehicle (distilled water, *p.o.*), GLUT (1 g/kg), EPOC containing 1.0 µg ethinylestradiol plus 5.0 µg levonorgestrel and EPOC plus GLUT, respectively, daily for 8 weeks. Results showed that the administration of EPOC caused glucose dysregulation and increased triglyceride-glucose index and visceral adiposity, but the body weight and liver weight were not affected. However, EPOC significantly decreased adipose lipid, G6PD and glutathione and increased glycogen synthesis, ADA, XO, uric acid, lipid peroxidation, lactate production and gamma-glutamyl transferase activity (GGT). On the other hand, EPOC increased hepatic lipid, ADA, XO, uric acid, lipid peroxidation and lactate production and decreased glycogen synthesis, G6PD and glutathione. Nevertheless, supplementation with glutamine attenuated these alterations. Collectively, the present results indicate that EPOC causes metabolically induced obesity which is associated with adipose dysfunction and hepatic metabolic disturbance. The findings also suggest that glutamine confers metabo-protection with corresponding improvement in adipose and hepatic metabolic function by suppression of ADA/XO activity and enhancement of G6PD-dependent antioxidant defense.

Key Words

- ▶ adipose function
- ▶ antioxidant defense
- ▶ estrogen-progestin
- ▶ hepatic function
- ▶ metabolic obesity

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Introduction

Overweight, obesity and type 2 diabetes mellitus are closely related health problems of global significance that are leading causes of cardiometabolic disease, particularly in women (Guzder *et al.* 2006, Rosamond *et al.* 2007). According to World Health Organization (WHO) report,

39% of the world's adults (40% women) were found to be overweight, while about 13% (15% women) were obese (<https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>). Although excess weight gain has been strongly associated with deleterious effects that

increase the risk of premature death, as well as chronic metabolic disorders, there is an emerging hypothesis that body weight or BMI alone may not fully reveal the metabolic risk of an individual (Visscher *et al.* 2001). This obesity 'paradox' was initially observed by Ruderman and co-workers in Metabolically Obese Normal Weight (MONW) women that displayed metabolic phenotypes including impaired insulin signaling, dyslipidemia and systemic inflammation, all of which are central to the development of metabolic syndrome (Ruderman *et al.* 1981, Conus *et al.* 2004, Carnethon *et al.* 2012). The MONW syndrome has been an understudied concept, despite its increasing prevalence over time, coupled with the lack of criteria for and difficulty of early diagnosis due to the normal body weight/BMI and young age of subjects. However, some studies including a recent study from our laboratory animals have shown that MONW individuals could be identified based on increased lipid profiles or the triglyceride-glucose (TyG) index which is a proven marker of insulin resistance (Lee *et al.* 2015, Olaniyi *et al.* 2019).

Excessive accumulation of fat in the abdominal region is a strong predictor of insulin resistance (IR); however, other factors such as genetic or environmental factors can predispose individuals to the development of this condition, since there are obese patients without insulin resistance as well as lean individuals who are resistant to insulin action (Lebovitz & Banerji 2005). In IR condition, adipose tissue function is impaired, which causes efflux of excess lipids into circulation from where deposition occurs in non-adipose tissues, particularly the liver (Crescenzo *et al.* 2015, Einer *et al.* 2018, Sergi *et al.* 2019). This hepatic lipid deposition has been documented as a strong predictor of non-alcoholic fatty liver disease (NAFLD), which can progress to liver disease (Wei *et al.* 2008, Feng *et al.* 2014). The severity of fatty liver disease has been previously associated with visceral fat accumulation and IR in both obese and non-obese subjects, suggesting that hepatic fatty infiltration in NAFLD may be influenced by visceral fat accumulation irrespective of body weight/BMI (Eguchi *et al.* 2006), although the mechanism remains unclear.

Combined oral contraceptive (COC), including estrogen-progestin oral contraceptive (EPOC), is the most popular effective means of birth control with over 100 million users worldwide (Moschos & Nitoda 2017, Rike *et al.* 2019). Its usage has been alternatively beneficial in the treatment of reproductive disorders such as dysmenorrhea and hormone replacement therapy for postmenopausal women. Despite the extensive clinical experience, many vascular and metabolic effects of COC steroids are under investigation. Changes in body weight are known to occur in some

women, although the association remains subclinical except in individuals with concomitant risk such as hypertension, cigarette smoking, atherosclerosis and diabetes among others (Africander *et al.* 2011). Available data show varying responses in body weight and visceral fat with ensuing IR and metabolic disturbances (Morin *et al.* 2008, Moschos & Nitoda 2017). In some experimental studies, COC treatments were reported to increase body weight and visceral adiposity, while other studies reported decreased or unchanged body weight despite evident increase in visceral adipose tissue mass and traditional markers of dysmetabolism (Gallo *et al.* 2004). This suggests that COC-induced IR is associated with increased visceral adiposity regardless of body weight gain. Understanding the pathophysiology that underlies MONW syndrome in COC users will not only provide valuable translation of the molecular and physiological significance of visceral adiposity, but will also inform clinical assessments of risk in MONW individuals with novel therapeutic measures that would protect women taking COCs including EPOC from the associated metabolic consequence.

Glutamine is a non-essential amino acid that is now known to be 'conditionally essential' in certain disease conditions. A number of studies in humans and animals have reported that glutamine confers beneficial effects on metabolic pathologies by improving insulin signaling, glucose homeostasis and regulation of gut microbiota. (Pratt *et al.* 2010, Cruzat *et al.* 2014, 2018, Jegatheesan *et al.* 2016, Olaniyi & Olatunji 2019). Despite the existing findings on dietary or pharmacological approaches to improve metabolic profile in women with metabolic syndrome, there is no specific nutritional/pharmacological recommendation for protection against metabolic disorder in the MONW sub-population. Therefore, our hypothesis that L-glutamine (GLUT) supplementation would be a potential therapeutic remedy against adipose dysfunction and excess hepatic lipid deposition that possibly accompany MONW in animals treated with EPOC by suppressing adenosine deaminase (ADA) activity and improving glucose-6-phosphate dehydrogenase (G6PD)-dependent antioxidant defense is worth studying.

Materials and methods

Animals

The investigation was conducted by following the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Review Board of the University of Ilorin, and every effort was made to minimize both the number of animals used

and their suffering. Adult female Wistar rats weighing between 150–180 g were obtained from the animal house of the College of Health Sciences, University of Ilorin (Ilorin, Nigeria). Rats had unlimited access to standard rat chow and tap water. After 1 week of acclimatization, the animals were randomly assigned to four groups ($n=6$ /group). Rats were maintained in the animal house under environmental conditions of temperature (22–26°C), relative humidity (50–60%) and 12 h light:12 h darkness cycle.

Treatment

Control group received vehicle (distilled water; *p.o.*), GLUT group received L-glutamine (1 g/kg; LOBA Chemie), EPOC group received COC containing 1.0 µg ethinylestradiol and 5.0 µg levonorgestrel (Wyeth-Ayerst) and EPOC+GLUT group received a combination of EPOC plus L-glutamine. The animals were treated daily for 8 weeks. Initial and final body weights were monitored, and the percentage of body weight gain was estimated.

Sample preparation

At the end of the treatment, the rats were anesthetized with sodium pentobarbital (50 mg/kg; *i.p.*). Blood was collected by cardiac puncture into heparinized tube and was centrifuged at 704 *g* for 5 min at room temperature. Plasma was stored frozen until needed for biochemical assay.

Liver weight (hepatic mass)

The liver was excised, cleared of adhering connective tissues, blotted and weighed immediately and corrected to body weight to eliminate variability.

Assessment of visceral adiposity

After dissection, visceral fat mass was assessed as previously described (Schiafone *et al.* 2017, Olaniyi & Olatunji 2019) by isolating and weighing the total perirenal, retroperitoneal and abdominal fat pad and corrected to body weight to eliminate variability.

Preparation of hepatic, adipose and pancreatic tissue homogenates

After dissection, each of the tissues was excised, cleared of adhering connective tissues and blotted. Thereafter, 100 mg of each tissue was carefully removed and homogenized with a glass homogenizer in cold phosphate buffer solution, centrifuged at 7826 *g* for 10 min at 4°C.

Glycemic and metabolic indices

Terminal fasting blood glucose (FBG) was determined after the rats had a 12-h overnight fast on the day of killing. Blood glucose levels were determined with a glucometer (ONETOUCH®-Life Scan). Insulin resistance was determined using the homeostasis model assessment for IR ($\text{HOMA-IR} = \text{FBG (mmol/L)} \times \text{fasting insulin (}\mu\text{U/L)} / 22.5$) (Hsing *et al.* 2003), while quantitative insulin sensitivity check index was used to determine insulin sensitivity; $\text{QUICKI} = 1 / (\text{Log (fasting insulin)} + \text{Log (fasting glucose)})$ (Katz *et al.* 2000). Metabolically induced obesity was assessed using the triglyceride-glucose index, which is expressed as $\text{TyG} = \text{Ln (TG (mg/dL)} \times \text{FPG (mg/dL)} / 2)$ (Lee *et al.* 2015, Olaniyi *et al.* 2019).

Biochemical assays

Plasma and pancreatic insulin

Plasma and pancreatic insulin concentration were determined using ELISA kits obtained from Ray Biotech. This method was based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule.

Lipid profile

Triglycerides (TG), total cholesterol (TC) and free fatty acid (FFA) were estimated in the adipose and hepatic tissue homogenates by standardized colorimetric methods using assay kits obtained from Randox. The quantification of the tissue concentration of TG, TC and FFA (non-esterified) were determined by coupled enzyme assays which result in colorimetric (570 nm) products proportional to the TG, TC and FFA present, respectively (Miller *et al.* 2016, Paul & Borah 2017).

Glycogen concentration and glycogen synthase activity

Adipose and hepatic glycogen concentration and glycogen synthase activity were determined as previously described (Vilkova 1982, Vardanis 1992, Bezborodkina *et al.* 2014) using standard non-enzymatic and enzymatic spectrophotometric methods, respectively, and reagents obtained from Oxford Biomedical Research.

Lipid peroxidation biomarker and antioxidant-dependent parameters

Malondialdehyde (MDA) is a marker of lipid peroxidation and was determined from adipose and

hepatic tissue homogenates by standard non-enzymatic spectrophotometric method using assay kits from Oxford Biomedical Research. This method involves the reaction of MDA in the sample with thiobarbituric acid (TBA) to generate a MDA-TBA adduct (Uchiyama & Mihars 1978), which was quantified spectrophotometrically, whereas glucose-6-phosphate dehydrogenase (G6PD) activity and glutathione (GSH) were determined using standard enzymatic and non-enzymatic spectrophotometric methods, respectively, with assay kits obtained from Oxford Biomedical Research. The G6PD activity spectrophotometric determination is based on the evaluation of absorbance at 340 nm given by NADPH formation; the quantitative evaluation is made by adding a precise amount of enzyme solution to an assay mixture containing the substrate (glucose-6-phosphate) and its cofactor NADP; the rate of NADPH generation is spectrophotometrically measured at wavelength of 340 nm (Beutler 1984). Whereas, GSH spectrophotometric determination was based on the oxidation of GSH in the sample by the sulfhydryl reagent 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5'-thio-2-nitrobenzoic acid (TNB), measured at 412 nm. The glutathione disulfide (GSSG) formed was recycled to GSH by glutathione reductase in the presence of NADPH (Rahman *et al.* 2006).

Lactate concentration and lactate dehydrogenase activity

Lactate concentration and lactate dehydrogenase (LDH) activity were determined in the adipose and hepatic tissue homogenates using standardized non-enzymatic and enzymatic colorimetric methods, respectively, and assay kit obtained from Randox. The colorimetric determination of lactate was based on the oxidation of lactate by lactate oxidase into pyruvate and hydrogen peroxide. The hydrogen peroxide is then detected with colorimetric probe. The reaction between the probe and hydrogen peroxide was catalyzed by horseradish peroxidase (Robergs *et al.* 2004) and quantified at 570 nm. Detection of LDH activity was based on the oxidation of NAD to NADH by LDH, which was specifically detected by colorimetric (450 nm) assay (Lu *et al.* 2008).

Gamma-glutamyl transferase

Gamma-glutamyl transferase (GGT) activities were determined from adipose and hepatic tissue homogenates as previously described (Phillip & Graham 1995, Young 2001) using standard spectrophotometric method and reagents obtained from Randox.

Adenosine deaminase activity, xanthine oxidase activity and uric acid concentration

From adipose and hepatic tissue homogenates, adenosine deaminase (ADA) and xanthine oxidase (XO) activities in addition to uric acid concentration were estimated by standard enzymatic and non-enzymatic colorimetric methods, respectively, using assay kits from Randox and in compliance with the manufacturers' assay procedures.

Data analysis and statistics

All data were expressed as means $\pm$ s.e.m. Statistical group analysis was performed with SPSS. One-way ANOVA was used to compare the mean values of variables among the groups. Bonferroni's test was used to identify the significance of pairwise comparisons of mean values among the groups. *P* values less than 0.05 were accepted as statistically significant.

Results

Effects of L-glutamine on body weight, visceral adiposity and liver weight in estrogen-progestin oral contraceptive-treated female rats

Administration of EPOC significantly increased visceral adiposity (16.43 ± 1.22 vs 9.62 ± 0.84 g/kg; $P < 0.05$) when compared with control group, this has been earlier documented to be central to the development of metabolic obesity (Stanhope *et al.* 2009, Adeyanju *et al.* 2018). However, EPOC did not significantly alter the body weight (26.28 ± 3.00 vs $28.95 \pm 2.30\%$) and liver weight/hepatic mass (11.50 ± 1.29 vs 9.78 ± 0.84 g/kg) when compared with control group. Besides, supplementation with glutamine decreased visceral adiposity (10.64 ± 1.40 vs 16.43 ± 1.22 g/kg; $P < 0.05$) when compared with EPOC-treated group (Fig. 1).

Effects of L-glutamine on glycemic and metabolic indices in estrogen-progestin oral contraceptive-treated female rats

The administration of EPOC increased HOMA-IR (8.90 ± 1.12 vs 4.07 ± 0.34 ; $P < 0.05$), which is an indicator of insulin resistance (Hsing *et al.* 2003). Similarly, EPOC increased plasma (49.61 ± 2.70 vs 23.31 ± 0.72 μ IU/mL; $P < 0.05$) and pancreatic (4.33 ± 0.22 vs 3.19 ± 0.11 μ IU/100 mg tissue; $P < 0.05$) insulin concentration, but decreased insulin sensitivity (0.43 ± 0.013 vs 0.51 ± 0.010 ; $P < 0.05$), as revealed by QUICKI (Katz *et al.* 2000), with no significant

Figure 1

Effects of L-glutamine (GLUT) on body weight (A), visceral adiposity (B) and liver weight (C) in estrogen-progestin oral contraceptive (EPOC)-treated female rats. EPOC increases visceral adiposity but not body weight and liver weight. However, glutamine supplementation attenuates the alterations. Data are expressed as mean $\pm$ S.E.M. $n = 6$. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test (* $P < 0.05$ vs control; # $P < 0.05$ vs EPOC).

change in glycemic level (4.15 ± 0.16 vs 3.82 ± 0.23) when compared with control group. It also increased the triglyceride-glucose index (11.14 ± 0.35 vs 9.17 ± 0.30 ; $P < 0.05$) which is a validated indicator of metabolically induced obesity (Lee *et al.* 2015, Olaniyi *et al.* 2019). However, supplementation with glutamine improved metabolically induced obesity (9.80 ± 0.32 vs 11.14 ± 0.35 ; $P < 0.05$), insulin sensitivity (0.49 ± 0.015 vs 0.43 ± 0.013 ; $P < 0.05$) and glucose homeostasis with corresponding decrease in plasma (30.13 ± 2.70 vs 49.61 ± 2.70 μ IU/mL; $P < 0.05$) and pancreatic (3.25 ± 0.18 vs 4.33 ± 0.22 μ IU/100 mg tissue; $P < 0.05$) insulin concentration when compared with EPOC-treated group (Fig. 2).

Effects of L-glutamine on adipose and hepatic lipid profile in estrogen-progestin oral contraceptive-treated female rats

Estrogen-progestin oral contraceptive decreased adipose TG (0.58 ± 0.01 vs 0.71 ± 0.01 mg/100 mg tissue; $P < 0.05$), TC (0.93 ± 0.05 vs 1.16 ± 0.03 mg/100 mg tissue; $P < 0.05$) and FFA (2.41 ± 0.07 vs 2.80 ± 0.05 mg/100 mg tissue; $P < 0.05$), which implies increased lipolysis, whereas increased hepatic TG (0.70 ± 0.03 vs 0.45 ± 0.02 mg/100 mg tissue; $P < 0.05$) and TC (1.70 ± 0.05 vs 1.38 ± 0.02 mg/100 mg tissue; $P < 0.05$) but not FFA (5.00 ± 0.33 vs 4.60 ± 0.02 mg/100 mg tissue) when compared with control group, and these result in hepatic lipid accumulation that possibly precedes fatty liver in obesity or metabolic disorders (Kitade *et al.* 2017). Nevertheless, glutamine supplementation significantly increased the lipid contents of adipose tissue (TG: 0.68 ± 0.01 vs 0.58 ± 0.01 mg/100 mg tissue, TC: 1.15 ± 0.08 vs 0.93 ± 0.05 mg/100 mg tissue and FFA: 2.76 ± 0.07 vs 2.41 ± 0.06 mg/100 mg tissue; $P < 0.05$) and decreased hepatic lipid (TG: 0.50 ± 0.04 vs 0.70 ± 0.03 mg/100 mg tissue and TC: 1.43 ± 0.04 vs 1.70 ± 0.05 mg/100 mg tissue; $P < 0.05$) compared to the EPOC-treated group (Fig. 3).

Effects of L-glutamine on adipose and hepatic glycogen concentration and glycogen synthase activity in estrogen-progestin oral contraceptive-treated female rats

Estrogen-progestin oral contraceptive increased adipose glycogen synthesis with corresponding increase in glycogen synthase activity (0.14 ± 0.007 vs 0.11 ± 0.006 μ g protein; $P < 0.05$) and glycogen content (0.51 ± 0.03 vs 0.38 ± 0.01 μ g/100 mg tissue; $P < 0.05$), causing adipose glycogen accumulation but decreased glycogen synthase

Figure 2

Effects of L-glutamine (GLUT) on glycemic and metabolic indices (A, B, C, D, E and F) in estrogen-progestin oral contraceptive (EPOC)-treated female rats. EPOC causes insulin resistance (HOMA-IR) and increases plasma and pancreatic insulin, but decreases insulin sensitivity (QUICKI). It also increases triglyceride-glucose index. However, glutamine supplementation attenuates the alterations. Data are expressed as mean $\pm$ S.E.M. $n = 6$. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test (* $P < 0.05$ vs control; # $P < 0.05$ vs EPOC).

activity (0.17 ± 0.01 vs 0.25 ± 0.01 μg protein; $P < 0.05$) and glycogen concentration (0.96 ± 0.04 vs 1.34 ± 0.05 $\mu\text{g}/100$ mg tissue; $P < 0.05$) in the hepatic tissue compared with control group. In addition, EPOC-treated group decreased adipose glycogen synthase activity (0.09 ± 0.005 vs 0.11 ± 0.006 μg protein; $P < 0.05$) without significant effect on glycogen content (0.36 ± 0.02 vs 0.38 ± 0.01 $\mu\text{g}/100$ mg tissue) when compared with the control group. However, supplementation with glutamine ameliorated adipose glycogen accumulation (glycogen content: 0.40 ± 0.02 vs 0.51 ± 0.03 $\mu\text{g}/100$ mg tissue and glycogen synthase activity: 0.12 ± 0.006 vs 0.14 ± 0.007 μg protein; $P < 0.05$) and hepatic glycogen depletion (glycogen content: 1.24 ± 0.08 vs 0.96 ± 0.04 $\mu\text{g}/100$ mg tissue and glycogen synthase: 0.22 ± 0.02 vs 0.17 ± 0.01 μg protein; $P < 0.05$) compared to the unsupplemented EPOC group (Fig. 4).

Effects of L-glutamine on adipose and hepatic uric acid concentration, adenosine deaminase and xanthine oxidase activity in estrogen-progestin oral contraceptive-treated female rats

Administration of EPOC increased adipose and hepatic ADA (adipose tissue: 0.048 ± 0.0013 vs 0.033 ± 0.0015 μg protein; $P < 0.05$) with correspondent increase in XO activity (adipose tissue: 0.12 ± 0.006 vs 0.08 ± 0.004 μg protein and hepatic tissue: 0.60 ± 0.02 vs 0.42 ± 0.02 μg protein; $P < 0.05$) and uric acid concentration (adipose tissue: 0.036 ± 0.0015 vs 0.020 ± 0.0012 $\mu\text{g}/100$ mg tissue and hepatic tissue: 0.067 ± 0.0020 vs 0.039 ± 0.0014 $\mu\text{g}/100$ mg tissue; $P < 0.05$) compared to the control group. Nonetheless, glutamine supplementation significantly reduced ADA (adipose tissue: 0.038 ± 0.0020 vs 0.048 ± 0.0013 μg protein

and hepatic tissue: 0.16 ± 0.010 vs 0.22 ± 0.012 μg protein; $P < 0.05$), XO (adipose tissue: 0.09 ± 0.008 vs 0.12 ± 0.006 μg protein and hepatic tissue: 0.49 ± 0.03 vs 0.60 ± 0.02 μg protein; $P < 0.05$) and uric acid concentration (adipose tissue: 0.027 ± 0.0020 vs 0.036 ± 0.0015 $\mu\text{g}/100$ mg tissue and hepatic tissue: 0.053 ± 0.003 vs 0.067 ± 0.0020 $\mu\text{g}/100$ mg; $P < 0.05$) in both tissues when compared with the EPOC-treated group. Besides, the hepatic but not adipose XO activity (hepatic tissue: 0.49 ± 0.03 vs 0.42 ± 0.02 $\mu\text{g}/100$ mg; $P < 0.05$) and uric concentration (hepatic tissue: 0.053 ± 0.003 vs 0.039 ± 0.0014 $\mu\text{g}/100$ mg; $P < 0.05$) were significantly higher in EPOC+GLUT-treated compared to the control group (Fig. 5).

Effects of L-glutamine on adipose and hepatic lactate production and gamma-glutamyl transferase in estrogen-progestin oral contraceptive-treated female rats

Estrogen-progestin oral contraceptive increased lactate production which correlated with elevated LDH (adipose tissue: 0.51 ± 0.030 vs 0.32 ± 0.020 μg protein and hepatic tissue: 0.28 ± 0.010 vs 0.20 ± 0.016 μg protein; $P < 0.05$) and lactate concentration (adipose tissue: 0.050 ± 0.0015 vs 0.040 ± 0.0020 mg/100 mg tissue and hepatic tissue: 0.17 ± 0.008 vs 0.11 ± 0.002 mg/100 mg tissue; $P < 0.05$) in both tissues when compared with the control group. Notwithstanding, concomitant supplementation with glutamine significantly reduced LDH activity (adipose tissue: 0.40 ± 0.020 vs 0.51 ± 0.030 μg protein and hepatic tissue: 0.22 ± 0.015 vs 0.28 ± 0.010 μg protein; $P < 0.05$) and lactate concentration (adipose tissue: 0.038 ± 0.0020 vs 0.050 ± 0.0015 mg/100 mg tissue and hepatic tissue: 0.13 ± 0.004 vs 0.17 ± 0.008 mg/100 mg tissue; $P < 0.05$) in

Figure 3

Effects of L-glutamine (GLUT) on adipose and liver lipid profile (A, B, C, D, E and F) in estrogen-progestin oral contraceptive (EPOC)-treated female rats. EPOC decreases adipose triglyceride (TG), total cholesterol (TC) and free fatty acid (FFA), whereas liver TG and TC but not FFA significantly increase. Nevertheless, supplementation with glutamine normalizes the changes. Data are expressed as mean $\pm$ s.e.m. $n = 6$. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test (* $P < 0.05$ vs control; # $P < 0.05$ vs EPOC).

Figure 4

Effects of L-glutamine (GLUT) on adipose and liver glycogen (gly) and glycogen synthase (GS) activity (A, B, C and D) in estrogen-progestin oral contraceptive (EPOC)-treated female rats. EPOC increases adipose glycogen and glycogen synthase activity, whereas liver glycogen and glycogen synthase activity significantly decrease, and these alterations are reversed following glutamine supplementation. Data are expressed as mean $\pm$ S.E.M. $n = 6$. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test (* $P < 0.05$ vs control; # $P < 0.05$ vs EPOC).

both tissues. Additionally, adipose but not hepatic LDH (adipose tissue: 0.40 ± 0.020 vs 0.32 ± 0.020 $\mu\text{g/g protein}$; $P < 0.05$) was significantly higher in EPOC+GLUT-treated compared with control group. Similarly, EPOC increased adipose GGT (0.34 ± 0.017 vs 0.27 ± 0.022 $\mu\text{g/g protein}$; $P < 0.05$) but did not significantly affect hepatic GGT (0.12 ± 0.015 vs 0.10 ± 0.010 $\mu\text{g/g protein}$) when compared to the control group. Nevertheless, the GGT activity decreased significantly in the adipose tissue when supplemented with glutamine (0.26 ± 0.020 vs 0.34 ± 0.017 $\mu\text{g/g protein}$; $P < 0.05$) compared with EPOC-treated group (Fig. 6).

Effects of L-glutamine on adipose and hepatic malondialdehyde (lipid peroxidation) and glucose-6-phosphate- and glutathione-dependent antioxidant defense in estrogen-progestin oral contraceptive-treated female rats

Administration of EPOC increased MDA (adipose tissue: 0.130 ± 0.009 vs 0.080 ± 0.008 $\mu\text{M}/100\text{ mg tissue}$ and hepatic tissue: 0.18 ± 0.008 vs 0.11 ± 0.002 $\mu\text{M}/100\text{ mg tissue}$; $P < 0.05$) and decreased G6PD activity (adipose tissue: 0.012 ± 0.0009 vs 0.015 ± 0.0010 $\mu\text{g/g protein}$ and hepatic tissue: 0.019 ± 0.001 vs 0.024 ± 0.0008 $\mu\text{g/g protein}$; $P < 0.05$) and GSH content (adipose tissue: 0.21 ± 0.009 vs 0.27 ± 0.005 $\mu\text{g}/100\text{ mg tissue}$ and hepatic tissue: 0.45 ± 0.02 vs 0.53 ± 0.01 $\mu\text{g}/100\text{ mg tissue}$; $P < 0.05$) in both tissues compared to the control group. These alterations (MDA, G6PD and GSH) were respectively attenuated by glutamine supplementation in both tissues ((adipose tissue: 0.090 ± 0.010 vs 0.130 ± 0.009 $\mu\text{M}/100\text{ mg tissue}$ and hepatic tissue: 0.14 ± 0.007 vs 0.18 ± 0.008 $\mu\text{M}/100\text{ mg tissue}$; $P < 0.05$), (adipose tissue: 0.0165 ± 0.0008 vs 0.0120 ± 0.0009 $\mu\text{g/g protein}$ and hepatic tissue: 0.023 ± 0.001 vs 0.019 ± 0.001 $\mu\text{g/g protein}$; $P < 0.05$) and (adipose tissue: 0.26 ± 0.006 vs 0.21 ± 0.009 $\mu\text{g}/100\text{ mg tissue}$ and hepatic tissue: 0.52 ± 0.02 vs 0.45 ± 0.02 $\mu\text{g}/100\text{ mg tissue}$; $P < 0.05$). However, hepatic but not adipose MDA concentration (0.14 ± 0.007 vs 0.11 ± 0.002 $\mu\text{M}/100\text{ mg tissue}$; $P < 0.05$) was significantly higher in EPOC+GLUT-treated compared to the control group (Fig. 7).

Discussion

The key finding from the present study is that glutamine supplementation protects against adipose dysfunction and hepatic metabolic disturbance with corresponding

Figure 5

Effects of L-glutamine (GLUT) on adipose and liver adenosine deaminase (ADA) activity, xanthine oxidase (XO) activity and uric acid concentration (A, B, C, D, E and F) in estrogen-progestin oral contraceptive (EPOC)-treated female rats. EPOC increases adipose and liver ADA, XO and uric acid, and these alterations are attenuated by glutamine supplementation. Data are expressed as mean $\pm$ S.E.M. $n = 6$. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test (* $P < 0.05$ vs control; # $P < 0.05$ vs EPOC).

Figure 6

Effects of L-glutamine (GLUT) on adipose and liver lactate (A and B), lactate dehydrogenase (LDH; C and D) and gamma-glutamyl transferase (GGT; E and F) in estrogen-progestin oral contraceptive (EPOC)-treated female rats. EPOC increases adipose and liver lactate, LDH activity and adipose but not liver GGT activity. However, these alterations are attenuated by glutamine supplementation. Data are expressed as mean $\pm$ S.E.M. $n = 6$. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test (* $P < 0.05$ vs control; # $P < 0.05$ vs EPOC).

improvement in metabolically induced obesity in EPOC-treated rats, by suppression of ADA/XO activity and enhancement of G6PD-dependent antioxidant defense. The results showed that EPOC causes metabolically induced obesity (MONW) with corresponding increase in visceral adiposity without significantly affecting the hepatic mass when compared with control group. Similarly, it disrupted glucose homeostasis by decreasing insulin sensitivity, a condition that resulted in hyperinsulinemia. Besides, EPOC decreased adipose lipid with an increase in glycogen synthesis but increased hepatic lipid with a decrease in glycogen synthesis. These changes were associated with increased adipose and hepatic ADA-dependent pro-inflammation, lipid peroxidation, lactate production, defective G6PD- and glutathione-dependent antioxidant defense and increased adipose but not hepatic GGT activity. However, supplementation with glutamine ameliorated these metabolic perturbations that accompany the administration of EPOC (Fig. 8).

The present results validated our recent and earlier findings that COC, especially estrogen-progestin

formulation, causes metabolically induced obesity (Olaniyi *et al.* 2019) in addition to insulin resistance, which is associated with disrupted glucose homeostasis, reduced insulin sensitivity and hyperinsulinemia (Olatunji *et al.* 2017a, Adeyanju *et al.* 2018, Michael & Olatunji 2018) respectively. Similarly, the present study provides evidence that EPOC-induced glucose dysregulation is not due to impaired pancreatic function nor insulin secretion but attributable to reduced insulin sensitivity which previous studies have largely associated with impaired insulin signaling (Pereira *et al.* 2017, Ferrannini *et al.* 2018). This perhaps triggers insulin resistance that precedes the development of metabolically induced obesity in animals treated with EPOC. In addition, the metabolically induced obesity observed in the present study is associated with increased fat mass in the visceral compartment, and this is an integral part of metabolic obesity as previously demonstrated (Stanhope *et al.* 2009, Adeyanju *et al.* 2018). However, supplementation with glutamine was found to improve insulin sensitivity, glucose homeostasis and normalize visceral adiposity, which is sufficient to

Figure 7

Effects of L-glutamine (GLUT) on adipose and liver malondialdehyde (MDA; A and B), glucose-6-phosphate dehydrogenase (G6PD) activity (C and D) and glutathione (GSH; E and F) in estrogen-progestin oral contraceptive (EPOC)-treated female rats. EPOC increases adipose and liver MDA and decreases G6PD activity and glutathione, which are ameliorated by glutamine supplementation. Data are expressed as mean $\pm$ S.E.M. $n = 6$. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test (* $P < 0.05$ vs control; # $P < 0.05$ vs EPOC).

Figure 8

Pathway showing the induction of metabolically induced obesity by estrogen-progestin oral contraceptive (EPOC) and the interaction with glutamine (GLUT) in the amelioration of EPOC-induced metabolic effects. ADA, adenosine deaminase; XO, xanthine oxidase; G6PD, glucose-6-phosphate dehydrogenase; UA, uric acid. A full color version of this figure is available at <https://doi.org/10.1530/JOE-19-0582>.

restore metabolic health in EPOC + GLUT-treated animals. This observation is consistent with previous studies (Cruzat *et al.* 2014, 2018, Jegatheesan *et al.* 2016) and the underlying mechanism worth probing.

It is not surprising that EPOC-induced insulin resistance increased lipolysis, leading to decreased adipose lipid profile including TG, TC and FFA and increasing lipid trafficking and ectopic deposition in non-adipose tissue especially the liver. Nonetheless, it is intriguing, despite this finding, to observe increased visceral adiposity which possibly contributes to metabolically induced obesity. Adipose tissue is responsible for fat storage including excess fat and its function as energy reservoir and endocrine tissue is a key factor in metabolic health. However, in insulin resistance-linked disorders, such as obesity, type 2 diabetes and other related metabolic syndromes, the white visceral adiposity increases, remodels and expands, leading to adipose dysfunction with consequent depletion of adipose triglyceride and other forms of lipids such as total cholesterol and free fatty acid. This may further aggravate systemic and hepatic insulin resistance (Haczeyni *et al.* 2015, Vishvanath & Gupta 2019), possibly accompanying metabolically induced obesity and contributing to the cardiac events usually reported in EPOC users (Morin *et al.* 2008, Haarala *et al.* 2009, Sitruk-ware & Nath 2013, Olatunji *et al.* 2017b). In addition, the increased adiposity is associated with elevated adipose glycogen content and glycogen synthase activity causing glycogen accumulation in the adipose tissue of the EPOC-treated animals. This is possibly due to redirection of glucose metabolic flux into glycogen synthesis in the adipose tissue, an observation that seems consistent with previous study by Ceperuelo-Mallafre

et al., who demonstrated excessive glycogen deposition in the adipose tissue that triggers inflammatory-related metabolic stress and affects cellular secretory profile in insulin resistant-obese subject (Ceperuelo-Mallafre *et al.* 2016). Therefore, the present findings suggest that adipose dysfunction with corresponding increased adiposity and glycogen accumulation may at least, in part, contribute to the development of metabolically induced obesity in EPOC-treated animals.

On the other hand, the administration of EPOC led to increased hepatic triglyceride and total cholesterol, causing hepatic lipid deposition, which has been previously documented to contribute to hepatic lipotoxicity in individuals with insulin resistance-related syndromes (Samuel *et al.* 2010, Olaniyi & Olatunji 2019). The excessive deposition of lipid without alteration in the level of the hepatic FFA possibly suggests that the EPOC-induced hepatic lipid accumulation is largely due to lipid influx into the liver, perhaps, resulting from insulin resistant adipose tissue. This effect may also be associated with impaired hepatic glycogen synthase activity that leads to decrease in hepatic glycogen content. An event similar to the previous reports that loss of hepatic glycogen synthesis diverts glucose toward fat synthesis, correlating with impaired hepatic insulin signaling and glucose disposal, and results in excess hepatic lipid that precedes hepatic steatosis (Obeid *et al.* 2000, Samuel *et al.* 2010, Williams *et al.* 2013) which is associated with metabolic obesity. However, glutamine, which is previously documented to improve insulin sensitivity (Jegatheesan *et al.* 2016, Cruzat *et al.* 2018), ameliorates EPOC-induced adipose dysfunction and hepatic lipid deposition.

Importantly, the administration of EPOC significantly increased adipose and hepatic ADA activity with subsequent increase in XO and uric acid concentration, a pro-inflammatory-engendered process that is pathologically triggered possibly by decreased circulating estradiol following treatment with EPOC. An earlier study from our laboratory animals has associated an increase in circulating uric acid to decreased plasma estradiol and impaired glucose uptake after treatment with estrogen-progestin formulation of COC (Olatunji *et al.* 2017a). Besides, ADA is a rate-limiting enzyme that deaminates intracellular pool of adenosine and deoxyadenosine, leading to uric acid production through XO activity. It is known to elicit a pro-inflammatory response and promote oxidative stress via the activity of XO, as reflected by elevated adipose and hepatic lipid peroxidation in this study, and at least in part contributes to adipose glycogen-induced pro-inflammatory metabolic signals and hepatic oxidative insults that accompany metabolically induced obesity in EPOC-treated animals. This is similar to previous studies including a recent study from our group which reported increased ADA and XO in metabolic-related disorders, particularly prediabetes, type 2 diabetes mellitus and cardiomyopathy among others (Feoli *et al.* 2014, Domingueti *et al.* 2016, Li *et al.* 2018, Olaniyi & Olatunji 2019). In addition, EPOC administration decreased house-keeping enzyme-G6PD- and GSH-dependent antioxidant defense when compared with control, which further reduced cellular resistance to oxidative stress, inducing cellular injuries as revealed by elevated lactate production and GGT in adipose tissue and lactate production but not GGT in hepatic tissue. Nevertheless, supplementation with glutamine decreased ADA-dependent pro-inflammation and lipid peroxidation with corresponding improvement of G6PD activity and GSH content in both tissues. This seems consistent with earlier studies that demonstrated beneficial effects of glutamine in metabolic pathologies including type 2 diabetes, cardiac hypertrophy and liver disease among others, and its beneficial effects have been linked to its anti-inflammatory and antioxidant properties (Grau *et al.* 2011, Million *et al.* 2013, Olaniyi & Olatunji 2019). Therefore, the present study suggests for the first time that glutamine supplementation improves adipose function and hepatic lipid deposition by suppression of ADA-dependent pro-inflammation and enhancement of G6PD- and GSH-dependent antioxidant defense. Nevertheless, this study is not without mechanistic limitation, particularly the molecular mechanism underlying the modulatory effects of glutamine in EPOC-treated animals. Regardless, the study in addition provides justification for a detailed focus

on the molecular mechanism underpinning the regulatory role of glutamine in EPOC users and the data generated could possibly impact safety in over 100 million women that use OC, particularly EPOC.

Conclusion

The present study demonstrates that metabolically induced obesity invoked by estrogen-progestin oral contraceptive is accompanied by adipose dysfunction and hepatic metabolic disturbance. The findings also suggest that glutamine confers metabo-protection with corresponding improvement in adipose and hepatic metabolic function by suppression of ADA/XO activity and enhancement of G6PD-dependent antioxidant defense. Therefore, glutamine supplementation could be employed as intervention against metabolically induced obesity, adipose dysfunction and metabolic-related liver disorders. The molecular mechanism underlying the regulatory role of glutamine in EPOC-treated animals/users should be explored in future studies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

K S O and L A O conceived and designed the research. K S O conducted the experiments. K S O and L A O contributed to the new reagents and analytical kits, analyzed and interpreted the data, drafted the manuscript and read and approved the final manuscript.

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