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Chronic ingestion of a Western diet increases O-linked- β -N-acetylglucosamine (O-GlcNAc) protein modification in the rat heart

H.M Medford^{a,b}, J.C. Chatham^c, and S.A. Marsh^a

^aProgram in Nutrition and Exercise Physiology, College of Pharmacy, Washington State University, Spokane, WA, USA

^bGraduate Program in Nutrition and Exercise Physiology, College of Pharmacy, Washington State University, Spokane, WA, USA

^cDivision of Molecular and Cellular Pathology, Department of Pathology, University of Alabama at Birmingham, Birmingham, AL, USA.

Abstract

Aims—Protein O-GlcNAcylation is both a nutrient sensing and cellular stress response that mediates signal transduction in the heart. Chronically elevated O-GlcNAc has been associated with the development of cardiac dysfunction at both the cellular and organ levels in obesity, insulin resistance and diabetes. Development of these pathologies is often attributed to diets high in saturated fat and sugar (a “Western” diet; WES) but a role for O-GlcNAc in diet-induced cardiac dysfunction has not been established. The aims of this study were to examine the effect of chronic consumption of WES on cardiac O-GlcNAcylation and investigate associations of O-GlcNAc with cardiac function and markers of cellular stress.

Main methods—Young male rats received either a control diet (CON; n=9) or WES (n=8) diet for 52 weeks.

Key findings—There was no evidence of cardiac dysfunction, advanced glycation endproduct (AGE) accumulation, pathological cardiac hypertrophy, calcium handling impairment, fibrosis or endoplasmic reticulum stress in WES hearts. However, cardiac O-GlcNAc protein, particularly in the higher molecular weight range, was significantly higher in WES hearts compared to CON ($P<0.05$). Protein levels of the enzymes that regulate O-GlcNAc attachment were not different between groups; thus, the increased O-GlcNAcylation in WES hearts appears to be due to increased nutrient availability rather than enzymatic regulation of cellular stress.

Significance—These data suggest that diets high in saturated fat and sugar may contribute to the adverse effects of metabolic syndrome and diabetes by an O-GlcNAc-mediated process and that this may occur in the absence of overt cellular stress.

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Corresponding Author: Susan A. Marsh, Program in Nutrition and Exercise Physiology, College of Pharmacy, Washington State University, PO Box 1495, Spokane, WA, 99210-1495, USA. Phone: +1-509-358 7718; Fax: +1-509-358 7627; samarsh@wsu.edu.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest.

Keywords

Western diet; saturated fat; O-GlcNAc; cardiac metabolism

INTRODUCTION

Obesity is reaching epidemic proportions in the United States (Ogden et al. 2006) resulting in increased incidence of metabolic syndrome, type 2 diabetes and associated cardiac pathologies, and unsustainable increases in healthcare costs (Centers for Disease Control and Prevention 2008). The obesity epidemic is undoubtedly the result of myriad lifestyle factors and is often associated with increased consumption of a diet high in simple carbohydrates and saturated fat, commonly termed the “Western” diet (WES) (Zhou et al. 2000; Kenchaiah et al. 2002; Peterson et al. 2004; Ogden et al. 2006). Indeed, evidence suggests that WES not only results in obesity but may also promote cardiac dysfunction (Wilson et al. 2007; Akki and Seymour 2009; Burgmaier et al. 2010).

O-linked- β -N-acetylglucosamine (O-GlcNAc) is a monosaccharide that is synthesized via the hexosamine biosynthesis pathway (HBP) and attaches to serine and threonine residues of nuclear and cytosolic proteins. This process is considered analogous to phosphorylation as a post-translational modification and mediator of signal transduction. However, while phosphorylation is mediated by hundreds of kinases and phosphatases, only two enzymes regulate protein O-GlcNAcylation: O-GlcNAc transferase (OGT) which catalyzes attachment of O-GlcNAc and O-GlcNAcase (OGA) that removes the O-GlcNAc moiety. As entry into the HBP can occur via fructose 6-phosphate, flux through the HBP and synthesis and attachment of O-GlcNAc are essentially considered to be a nutrient sensing process. Indeed, O-GlcNAc levels are increased with high levels of extracellular substrates such as glucose, and are upregulated with diabetes (Fulop et al. 2007b; Marsh et al. 2011) and 12 weeks consumption of a high fat diet (Li et al. 2005). Interestingly, O-GlcNAc protein is also increased with cellular stresses, such as oxidative stress and heat shock (Zachara et al. 2004), but this does not appear to be mediated by substrate availability as protein O-GlcNAcylation is also upregulated with glucose deprivation, aging and Alzheimer’s disease (Griffith and Schmitz 1995; Fülöp et al. 2008; Taylor et al. 2008).

Despite the known nutrient sensing and cell stress responses of protein O-GlcNAcylation, the effect of long-term dietary manipulation on in vivo cardiac O-GlcNAc levels has not yet been established. Therefore, the goals of this study were to examine the effect of chronic consumption of WES on cardiac O-GlcNAcylation and investigate associations of O-GlcNAc with cardiac function and markers of cellular stress.

MATERIALS AND METHODS

Study protocol

This protocol was approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham. Four week old, male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were housed two per cage, maintained on a 12:12 hour light:dark cycle and provided with food and tap water ad libitum. Animals were randomized to receive control (CON; 12% kcal fat/19% protein/69% carbohydrate; 3.87 kcal/g; n=9) or Western (WES; 40/15/45%; 4.62 kcal/g; n=8) diets for 52 weeks; these diets have been used previously by our lab (Marsh et al. 2009) and were prepared and analyzed by TestDiet (Richmond, IN). Diets are not isocaloric; saturated fat content was higher in WES (10%) compared to CON (0.4%) and the contribution of simple sugars to the carbohydrate was also different between diets with a higher level of sucrose in WES (31% vs 17% w/w).

Fasting blood analysis

Fasting whole blood samples were collected from the retro-orbital plexus under light isoflurane anesthesia early in the light cycle; glucose and glycated hemoglobin (HbA1c) levels were measured in whole blood using the Accu-Chek Advantage (Roche Diagnostics, Basel, Switzerland) and DCA2000+ (Bayer Healthcare, Elkhart, IN) analyzers respectively. Blood for the remaining analyses was collected at sacrifice from the inferior vena cava after an overnight fast. A standard biochemistry panel was analyzed in rat plasma using the VetScan Chemistry Analyzer (Abaxis, Union City, CA).

Hemodynamic measurements

Rats were anesthetized with 2% isoflurane and a 1.5F pressure catheter transducer (Millar Instruments, Houston, Texas) was inserted into the carotid artery and high-fidelity aortic and LV pressures were recorded. Augmentation index, a measure of arterial stiffness, was calculated as the height of the reflected aortic pressure wave as a percentage of pulse pressure using customized software (SphygmoCor 8.0, AtCor Medical, Sydney, Australia).

Immunoblotting

LV tissue was homogenized in T-PER buffer (Thermo Scientific, Rockford, IL) containing 3% protease inhibitor cocktail, 1 mM sodium orthovanadate and 20 mM sodium fluoride and lysed for 60 min on ice; 0.2 v/v% (20mM stock) of PUGNac was added to prevent the removal of O-GlcNAc as previously described (Laczy et al. 2010) Tissue homogenates (10% w/v) were centrifuged at 14,000 g for 10 min and the protein concentration of the supernatant was assessed using Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA). Proteins were electrophoresed either on 6% SDS-polyacrylamide gels and then transferred to PVDF membranes or on 10% SDS-polyacrylamide gels and then transferred to nitrocellulose membranes. Protein O-GlcNAc levels were determined on nitrocellulose membranes by immunoblot analysis using the anti-O-GlcNAc antibody CTD110.6 (Thermo Fisher Scientific, Rockford, IL). PVDF blots were probed with primary antibodies for advanced glycation endproducts (AGE; Abeam, Cambridge, MA), glucose-regulated protein (GRP) 78 (Abeam), phosphorylated and total inositol-requiring enzyme 1 (pIRE1, IRE1; Abeam), fibronectin (Abeam), α -Actin (Abeam), SERCA2 (Santa Cruz Biotechnology, Santa Cruz, CA), atrial natriuretic peptide (ANP, Abeam), OGA (L-14; Santa Cruz Biotechnology), or OGT (DM-17; Sigma, St. Louis, MO) diluted in 1% casein and phosphate-buffered saline (PBS) buffer overnight at 4°C. Calsequestrin (Abeam) was used as an internal loading control. After incubation with appropriate secondary antibodies, blots were visualized with enhanced chemiluminescence (Thermo Fisher Scientific). Densitometric analysis was performed using ImageJ software (National Institutes of Health, Bethesda, MD).

Statistical analysis

A one-tailed Students' t-test was used to determine differences between groups. Values are presented as mean $\pm$ SEM and significance was established at $P < 0.05$.

RESULTS

Fasting blood glucose was not different between groups (Table 1); however, glycated hemoglobin (HbA1c) was significantly higher in WES compared to CON ($P < 0.05$), indicating impaired blood glucose control over an extended period of time. However, the observed values were within normal ranges for both groups. Several blood chemistry values were also different between groups (Table 1), including lower indices of renal (BUN:creatinine) function, albumin and several electrolytes in WES. Unexpectedly, there

were no significant differences between diets in serum cholesterol or triglycerides between groups ($P>0.05$).

After 52 weeks of feeding, there were no differences between diets in body weight or size or organ weights (Table 2). Surprisingly, systolic (+dP/dt) and diastolic (−dP/dt, tau) cardiac function was not different between groups (Table 3), although animals on the WES diet had a higher resting heart rate than CON ($P<0.05$). Augmentation index, a measure of arterial stiffness, was also not different between groups.

Consumption of a Western diet can lead to a metabolic derangement in the heart with no change in circulating blood glucose levels (Wilson et al. 2007). In LV tissue homogenates, there were no differences between diets in accumulation of advanced glycation endproducts (AGE) or development of fibrosis (fibronectin) (Figure 1A-C). Markers of endoplasmic reticulum (ER) stress, indicated by glucose regulated protein 78 (GRP78/BiP) and phosphorylated and total inositol-requiring enzyme 1 (IRE1), were not different between diets (Figure 1D-F). Calcium handling, as indicated by SERCA2, were not different between groups. Pathological cardiac hypertrophy, indicated by increased markers of the fetal gene program (atrial natriuretic peptide (ANP) and α -Actin) was not observed in WES compared to CON hearts (Figure 2).

Although flux through the HBP was not measured here, the synthesis and attachment of O-GlcNAc to proteins is a nutrient- and stress-sensitive process that alters cell signaling. Despite the lack of effect of WES on AGE, fibrosis or ER stress, we hypothesized that chronic consumption of saturated fat and sugar in the WES group would result in increased cardiac O-GlcNAcylation. As seen in Figure 3, overall O-GlcNAc levels were significantly higher in WES hearts compared to CON ($P<0.05$). Since densitometry of the entire sample lane is dominated by the intense immunoreactive bands at ~60 and 38 kDa, densitometry was also performed over the high, mid and low molecular weight ranges as indicated in Figure 3A. In the higher molecular weight range, O-GlcNAc levels were significantly higher in WES compared to CON ($P<0.05$; Figure 3B) and there was a trend towards higher levels in the lower molecular weight range ($P=0.07$; Figure 3C). OGT and OGA regulate the attachment and removal of the O-GlcNAc moiety, respectively. We did not observe any significant differences in OGT or OGA protein between groups (Figure 3D-E).

DISCUSSION

In the current study, we found that chronic (12 months) consumption of a diet high in saturated fat and sugar (a Western diet) increased protein O-GlcNAcylation in the heart, particularly in the higher molecular weight range compared to age-matched animals that consumed a control diet; this change was not associated with changes in regulatory enzymes (OGT, OGA), or evidence of cellular stress, pathological cardiac hypertrophy, impaired calcium handling, or cardiac dysfunction. These data suggest that the increase in O-GlcNAc was a result of increased nutrient and substrate availability and resultant increased flux through the HBP rather than cellular stress or alterations in OGT or OGA protein. This is the first study, to our knowledge, to demonstrate a long-term effect of diet on cardiac O-GlcNAc. Diets high in saturated fat and sugar have long been associated with metabolic syndrome and type 2 diabetes as evidenced by increases in adiposity, obesity, blood glucose, plasma insulin, insulin resistance and blood pressure (Li et al. 2005; Demigne et al. 2006; Esmailzadeh et al. 2007; Hartvigsen et al. 2007; Wilson et al. 2007; Lutsey et al. 2008). As chronic increases in cardiac O-GlcNAc levels are associated with cellular dysfunction, these findings suggest that the Western diet may exert detrimental effects via O-GlcNAc-dependent mechanisms. Additionally, the increased protein O-GlcNAcylation seen here is consistent with increases seen in type 2 diabetic hearts and isolated primary cardiomyocytes

(Fulop et al. 2007a; Marsh et al. 2011). This is an intriguing finding, as it suggests that a Western diet alone may induce similar changes to those seen in type 2 diabetes in the absence of significant hyperglycemia.

Nutrient availability, cellular stress and/or an altered balance between OGT and OGA enzyme levels or activity modulate O-GlcNAc synthesis and attachment (Zachara et al. 2004; Taylor et al. 2008). The Western diet used in our previous (Marsh et al. 2009) and current studies was high in sucrose; as sucrose can be broken down to form glucose or fructose and directly enter the hexosamine biosynthesis pathway and increase the substrate for O-GlcNAc, and sucrose feeding alone is detrimental to cardiovascular function (Davidoff et al. 2004), we suspect that the sucrose component of the diet was primarily responsible for the elevated O-GlcNAc levels we observed. The higher cardiac O-GlcNAc levels in WES in the absence of high circulating blood glucose is not necessarily surprising as HbA1c levels were significantly higher in WES (Table 1) which is indicative of poor long term glucose control, although the observed values were within healthy ranges. While we did not find evidence of ER stress in WES hearts, differences in plasma markers of renal function, albumin and electrolytes suggest a systemic detrimental effect of WES; this, in turn, may be the underlying cause of the elevated O-GlcNAc protein. We did not measure oxidative stress, plasma free fatty acids, or insulin resistance in this study due to plasma and tissue sample limitations; however, these are typically associated with higher O-GlcNAcylation and would be expected to be elevated in WES. We recently demonstrated that fatty acid oxidation was increased in response to increased O-GlcNAcylation in an isolated heart model with glucosamine supplementation (Laczy et al. 2011); we therefore hypothesize that a similar response occurred in our animals due to the fact that O-GlcNAc was increased through increased substrate availability. The enzymes OGT and OGA also regulate O-GlcNAc attachment to proteins. The lack of change of OGT and OGA in WES in the face of increased O-GlcNAc further supports the hypothesis that the higher O-GlcNAc was most likely due to increased nutrient availability and subsequent flux through the HBP rather than alterations in enzyme abundance or activity.

The notion that a Western diet is deleterious to cardiac function is based on studies that reported somewhat equivocal findings, such as derangements in substrate utilization and abnormalities in expression of genes that regulate cardiac hypertrophy in Western fed animals in the presence (Wilson et al. 2007; Akki and Seymour 2009) and absence (Burgmaier et al. 2010) of cardiac dysfunction. Studies that utilize a Western diet are also often confused with those that utilize a diet high in saturated fat with no change in sugar content which have shown a protective effect of saturated fat on the heart (Okere et al. 2005; Berthiaume et al. 2010). Therefore, it is perhaps not entirely surprising that we did not find any differences between diets in cardiac function or arterial stiffness after 52 weeks of feeding (Table 3) and the inconsistent findings between studies may be due to the differences in rat strains and dietary composition. It is also important to note that the lack of overt dysfunction at the whole heart level does not necessarily indicate a lack of subtle changes in cardiomyocyte function. Chronic increases in O-GlcNAc are associated with various pathologies including impaired excitation-contraction coupling and cardiomyocyte relaxation (Hu et al. 2005; Fulop et al. 2007b) in addition to vascular dysfunction (Lima et al. 2009; Lima et al. 2011); indeed, we recently demonstrated that O-GlcNAc impairs the response to hypertrophic stimuli in cardiomyocytes harvested from both diabetic and non-diabetic hearts (Marsh et al. 2011). This is an important finding, as cardiac hypertrophy is an early defense mechanism of the myocardium against physical insult or injury, and blunting of this response could cause heart failure to occur much more rapidly than usual. The significantly higher O-GlcNAc levels in the higher molecular weight proteins seen in Figure 3 are consistent with our recent work in type 2 diabetic hearts (Fulop et al. 2007b). While O-GlcNAc modified proteins were not identified in this study, others have shown that myosin

heavy chain, a high molecular weight contractile protein, is O-GlcNAcylated in the heart and that this is associated with reduced calcium sensitivity (Ramirez-Correa et al. 2008). It is also interesting to note that calcium handling proteins such as SERCA and the ryanodine receptor are also high molecular weight proteins although they have not yet been identified as being O-GlcNAc modified. Elevated O-GlcNAc is associated with decreased SERCA protein in diabetic hearts (Hu et al. 2005). In this study however, we observed higher O-GlcNAc levels in WES hearts with no change in SERCA (Figure 2), which may simply be a reflection of the euglycemia exhibited by WES animals. Interestingly, the SERCA regulator phospholamban is O-GlcNAcylated in diabetic hearts (Yokoe et al. 2010) which raises the possibility that SERCA activity is altered in WES hearts in the absence of any change in protein levels (Wold et al. 2005). The higher O-GlcNAc in WES hearts with no differences between diets in cardiac and vascular function may also be due to a confounding effect of aging as older hearts typically exhibit increased O-GlcNAc levels (Fulop et al. 2008) and impaired contractile function (Capasso et al. 1983). The lack of difference between diets in AGE, fibronectin and ER stress markers also suggest that a ceiling effect may have been reached with aging, potentially negating any effect of WES on these indices. A time course study would provide valuable additional insight into whether O-GlcNAc was temporally associated with WES-induced dysfunction; unfortunately, this was not possible due to the substantial resources required. However, current work in our lab is investigating the role of WES on cardiac O-GlcNAc and cell signaling and involve shorter periods of feeding and we hope that this will help elucidate the mechanisms underlying the changes we observed here.

CONCLUSION

To our knowledge, this is the first study to demonstrate an *in vivo* effect of a high saturated fat and simple sugar Western diet on O-GlcNAc levels in the heart. O-GlcNAc attachment is a transient, reversible process and chronic O-GlcNAc levels are associated with impaired cellular function and signaling, although there is increasing evidence that O-GlcNAcylation induces epigenetic changes (Hanover 2010). The HBP is known to be a nutrient sensing pathway and flux through this pathway increases O-GlcNAc synthesis and attachment; however, we do not know if dietary modification will reverse the elevated O-GlcNAc observed in this study. Increases in O-GlcNAcylation similar to those observed here are seen in disease states such as type 2 diabetes and cardiovascular disease; however, in chronic and/or severe disease the concomitant pathology may not be reversible. Thus, if the increased cardiac O-GlcNAc levels seen with the Western diet and in conditions such as insulin resistance and diabetes are irreversible and heritable, this will have profound ramifications for the health of future generations. Further studies are therefore clearly warranted to better elucidate the relationship between diet and O-GlcNAc in the heart.

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Figure 1.

Markers of advanced glycation endproducts (AGE) and fibrosis (fibronectin) (A-C) and endoplasmic reticulum stress (D-F) in the left ventricle of rats that consumed a control (CON) or western (WES) diet for 12 months (representative blots from a total of n=6 animals per group); calsequestrin was used as an internal loading control. GRP78/BiP, glucose regulated protein 78; pIRE1 and IRE1, phosphorylated and total inositol-requiring enzyme 1.

Figure 2. Representative western blots (A) and densitometric analyses of protein levels of SERCA2, α -Actin, ANP, and Calsequestrin (B-D) in control (C, CON) or western (W, WES) fed mouse hearts. Densitometry values were normalized to the protein loading control, Calsequestrin. n=6 per group.

Figure 3.

Representative western blots (A) and densitometric analyses of protein levels of O-GlcNAc using analysis of the entire lane (B) or high, mid and low molecular weights (C), in addition to O-GlcNAc transferase (OGT)(D) and O-GlcNAcase (OGA)(E) in control (C, CON) or western (W, WES) fed mouse hearts. Densitometry values were normalized to the protein loading control, calnexin. n=6 per group. * P<0.05 vs CON.

Table 1

Fasting blood and plasma biochemical parameters

	Control (n=9)	Western (n=8)
Blood glucose (mmol/l)	6.98 ± 0.32	6.58 ± 0.33
HbA1c (%)	3.48 ± 0.06	3.75 ± 0.07 *
Blood urea nitrogen (mg/dL)	13.2 ± 0.7	10.8 ± 0.9 *
Creatinine (mg/dL)	0.39 ± 0.02	0.39 ± 0.02
BUN: Creatinine	34.2 ± 1.6	27.8 ± 1.5 *
Amylase (U/L)	709 ± 25	609 ± 16 *
Lipase (IU/L)	10.5 ± 0.3	10.4 ± 0.3
Alanine aminotransferase (U/L)	66 ± 9	52 ± 6
Calcium (mg/dL)	10.26 ± 0.16	9.84 ± 0.11 *
Sodium (mg/dL)	135 ± 2	135 ± 1
Potassium (mg/dL)	8.05 ± 0.5	6.3 ± 1.5 *
Total protein (mg/dL)	6.3 ± 0.1	5.9 ± 0.1 *
Albumin (g/dL)	2.99 ± 0.04	2.83 ± 0.06 *
Cholesterol (mg/dL)	103 ± 11	86 ± 4
Triglycerides (mg/dL)	156 ± 15	153 ± 15
Osmolality	287 ± 5	286 ± 3

Values are mean ± SEM.

* P<0.05 vs Control diet.

Table 2

Body and organ weights

	Control (n=9)	Western (n=8)
Body weight (g)	806 ± 48	852 ± 36
Tibia length (mm)	51.6 ± 0.8	53.1 ± 0.6
LV wt/TL (mg/mm)	25.65 ± 0.77	23.83 ± 1.11
Kidney wt/TL (mg/mm)	38.2 ± 1.2	38.1 ± 1.3
Lung wt/TL (mg/mm)	2.45 ± 0.14	2.74 ± 0.14

Values are mean ± SEM. TL, tibia length; LV, left ventricle.

Table 3

Cardiac function and arterial stiffness

	Control (n=9)	Western (n=8)
MAP (mmHg)	128.6 ± 5.0	129.8 ± 4.2
Heart rate (beats/min)	330 ± 10	364 ± 13*
+dp/dt (mmHg/s)	9171 ± 492	9273 ± 552
−dp/dt (mmHg/s)	−8765 ± 495	−9445 ± 498
Tau (ms)	9.57 ± 0.65	8.41 ± 0.47
Augmentation index (%)	39.0 ± 4.1	37.2 ± 5.6

Values are mean ± SEM. MAP, mean arterial pressure; +dp/dt and −dp/dt, maximal and minimal change in pressure overtime, respectively.