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RAPID COMMUNICATION

Glucose Uptake and Triacylglycerol Synthesis Are Increased in Barth Syndrome Lymphoblasts

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Abstract Barth syndrome (BTHS) is an X-linked genetic disease resulting in loss of cardiolipin (Ptd_2Gro). Patients may be predisposed to hypoglycemia and exhibit increases in whole-body glucose disposal rates and a higher fat mass percentage. We examined the reasons for this in BTHS lymphoblasts. BTHS lymphoblasts exhibited a 60% increase ($p < 0.004$) in 2-[1,2-^{3}H(*N*)]deoxy-D-glucose uptake, a 40% increase ($p < 0.01$) in glucose transporter-3 protein expression, an increase in phosphorylated-adenosine monophosphate kinase (AMPK) and a 58% increase ($p < 0.001$) in the phosphorylated-AMPK/AMPK ratio compared to controls. In addition, BTHS lymphoblasts exhibited a 90% ($p < 0.001$) increase in D-[U-^{14}C] glucose incorporated into 1,2,3-triacyl-*sn*-glycerol (TAG) and a 29% increase ($p < 0.025$) in 1,2-diacyl-*sn*-glycerol acyltransferase-2 activity compared to controls. Thus, BTHS lymphoblasts exhibit increased glucose transport and increased glucose utilization for TAG synthesis. These results may, in part, explain why BTHS patients exhibit an increase in whole-body glucose disposal rates, may be predisposed to hypoglycemia and exhibit a higher fat mass percentage.

Keywords Cardiolipin · Metabolism · Glucose transport · Triacylglycerol synthesis · Barth syndrome

Abbreviations

BTHS	Barth syndrome
TAZ	Tafazzin
Ptd_2Gro	Cardiolipin
2-[^{3}H]DG	2-[1,2-^{3}H(*N*)]deoxy-D-glucose
GLUT1	Glucose transporter-1
GLUT3	Glucose transporter-3
DAG	1,2-Diacyl-*sn*-glycerol
DGAT-2	1,2-Diacyl-*sn*-glycerol acyltransferase-2
TAG	1,2,3-Triacyl-*sn*-glycerol
PtdGro	Phosphatidylglycerol
PtdCho	Phosphatidylcholine
PtdEtn	Phosphatidylethanolamine
PtdSer/PtdIns	Phosphatidylserine/phosphatidylinositol

Introduction

Ptd_2Gro is a key phospholipid required for mitochondrial energy production [1, 2]. BTHS is a rare X-linked genetic disease caused by mutations in the *TAZ* gene [3]. The *TAZ* gene produces the enzyme tafazzin that transfers fatty acyl groups from phospholipids such as phosphatidylcholine (PtdCho) to produce primarily unsaturated tetra-acyl species of Ptd_2Gro [4]. In BTHS, dramatically reduced levels of Ptd_2Gro and tetralinoleoyl-Ptd_2Gro are observed [3]. Historically regarded as a cardiac disease, BTHS is now considered a multi-system disorder. A previous study determined that BTHS patients exhibited a 43% greater

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insulin-stimulated glucose disposal rate per kilogram fat-free mass and a higher fat mass percentage compared to age-matched controls [5]. The mechanism for these effects was unknown. Here we show that glucose uptake and its utilization for TAG synthesis is increased in BTHS lymphoblasts.

Materials and Methods

All cell culture reagents and PCR primers were obtained from Invitrogen (Burlington, ON, Canada), and culture flasks and dishes from Corning, Inc. (Corning, NY, USA). Lipid standards were from Serdary Research Laboratories (Englewood Cliffs, NJ, USA). Thin-layer chromatography plates (silica gel G, 0.25-mm thickness) were obtained from Fisher Scientific (Winnipeg, Manitoba, Canada). Ecolite(+)^{TM} Liquid Scintillation was obtained from ICN Biochemicals (Montreal, Quebec, Canada). Unlabeled lipids and fatty acid-free bovine serum albumin (BSA) were from Sigma–Aldrich (Oakville, ON, Canada). 2-[1,2-^{3}H(*N*)]Deoxy-D-glucose (2-[^{3}H]DG), D-[U-^{14}C]glucose, [1-^{14}C]oleoyl-CoA and [1-^{14}C]acetate were obtained from PerkinElmer (Woodbridge, ON, Canada) or American Radiolabeled Chemicals (St. Louis, MO, USA). Primary and secondary antibodies for GLUT1 (sc-1603), GLUT3 (sc-74399), pAMPK$^{(Thr172)}$ (sc-33524), and AMPK (sc-398861) were from Santa Cruz Biotechnology Inc. (Dallas, TX, USA). Primary and secondary antibodies for cyclophilin B were from abcam (ab178397; Cambridge, MA, USA). Western blotting analysis system was from GE Healthcare Life Sciences (Missisauga, ON, Canada). All other biochemicals were certified ACS grade and were from Fisher Scientific (Winnipeg, Canada).

Epstein-Barr virus-transformed BTHS lymphoblasts from 4 to 9 year old males and male age-matched control lymphoblasts were obtained from Dr. Richard Kelly (John Hopkins University, Baltimore, MD, USA) and Coriell Institute for Medical Research (Camden, NJ. USA). Cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum (FBS) and 1% antimycotic and antibiotic solution. Cells were incubated at 37 °C in 5% CO_2 until required for studies.

Glucose uptake assay using 0.1 mM 2-[^{3}H]DG (0.5 μC/ml) was performed as described previously [6]. For determination of glycerolipid synthesis, cells were incubated with 0.1 mM D-[U-^{14}C]glucose (10 μCi/ml) for 24 h, and neutral lipids [1,2-diacyl-*sn*-glycerol (DAG); TAG] and glycerophospholipids were separated and radioactivity determined as previously described [7]. In some experiments, cells were incubated for 24 h with 0.1 μM [1-^{14}C] acetate (10 μCi/ml) and radioactivity incorporated into TG determined as above.

Western blot analysis was performed as described [8]. CL mass and species composition were determined as described [9]. DGAT-2 enzyme activity was determined using [1-^{14}C]oleoyl-CoA as previously described [8]. Protein was determined by the Bradford method using the Bio-Rad Bradford protein assay M kit [10]. All data were expressed as mean or mean ± standard deviation. Differences between two means were analyzed by Student's *t* test. $p < 0.05$ was considered to be statistically significant.

Results and Discussion

Age-matched control and BTHS lymphoblasts were incubated with 2-[^{3}H]DG and uptake into cells determined. BTHS lymphoblasts exhibited a 60% increase ($p < 0.004$) in 2-[^{3}H]DG uptake compared to age-matched control cells (Fig. 1a). The major detectable lymphoblast Ptd_2Gro molecular species for the control and BTHS lymphoblasts used in this study are shown in Table 1. In BTHS lymphoblasts, total Ptd_2Gro mass was reduced to 5% of control cells. In addition, tetralinoleoyl-Ptd_2Gro $(C18:2)_4$ levels in BTHS lymphoblasts were reduced to 0.7% of controls. Lymphoblasts express both GLUT1 and GLUT3 glucose transporter proteins, and expression of these may be regulated differently [11–13]. The GLUT1 protein level was unaltered in BTHS lymphoblasts compared to controls (Fig. 1b). In contrast, GLUT3 protein levels were increased 40% ($p < 0.01$) in BTHS lymphoblasts versus controls (Fig. 1c). A previous study showed that knockdown of tafazzin in neonatal ventricular fibroblasts increased phosphorylation and activation of AMPK [14]. AMPK activation may stimulate glucose uptake through increased GLUT3 expression [15]. P-AMPK was elevated approximately 100% (Fig. 2a), and the ratio of pAMPK$^{(Thr172)}$ to AMPK increased 58% ($p < 0.05$) in BTHS cells compared to control cells, which would explain the increased GLUT3 expression in BTHS lymphoblasts. If an increase in glucose transport is mirrored in other tissues, this may, in part, explain why BTHS patients exhibit increased rates of glucose disposal and may be predisposed to hypoglycemia [5, 16]. The increased rate of glucose disposal observed in BTHS patients is likely due to the requirement for alternative sources of adenosine triphosphate (ATP), as mitochondrial oxidative phosphorylation is greatly compromised due to loss of Ptd_2Gro. A previous study indicated that the basal extracellular acidification rate in cardiomyocytes from tafazzin knockdown mice was higher than that in controls, consistent with an increased reliance on glycolysis [17]. In that study it was hypothesized that the metabolic shift away from aerobic respiration and toward glycolysis in Ptd_2Gro-deficient cardiomyocytes was a compensatory mechanism

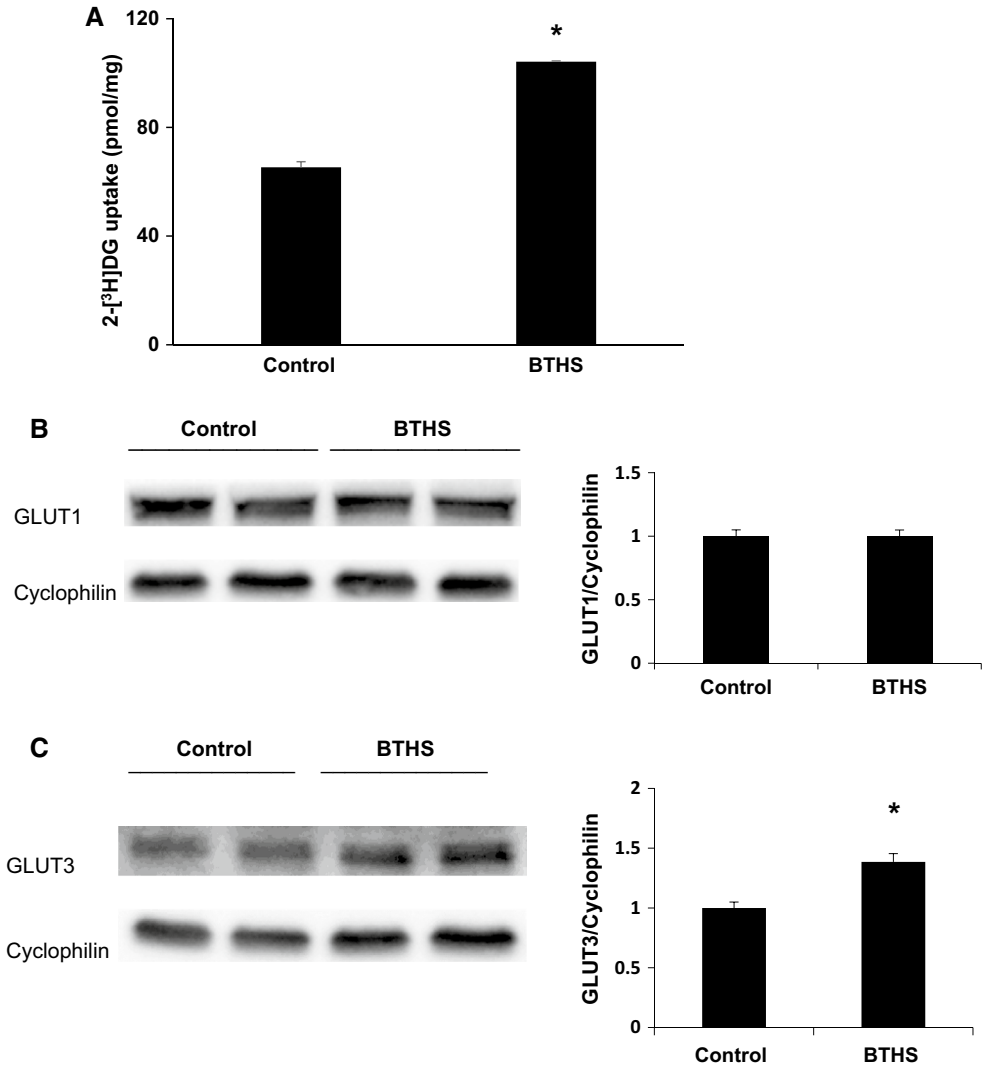

Fig. 1 2-[^{3}H]DG incorporation and GLUT1 and GLUT3 expression in control and BTHS lymphoblasts. **a** Control and BTHS lymphoblasts were incubated with 0.1 mM 2-[^{3}H]DG for 10 min, and radioactivity incorporated into cells was determined. *$p < 0.01$, $n = 3$. GLUT1 (**b**) and GLUT3 (**c**) expression in control and BTHS lymphoblasts was determined. Representative Western blots are depicted. *$p < 0.01$, $n = 3$

Table 1 The Ptd_2Gro content and major molecular species composition in control and BTHS lymphoblasts

	Control	BTHS
Total Ptd_2Gro (ng × 10^6 cells)	35.88	1.83
	% Total	% Total
Ptd_2Gro $(C18:2)_3(C18:3)$	7.87	43.99
Ptd_2Gro $(C18:2)_4$	21.76	4.23
Ptd_2Gro $(C18:2)_3(C18:1)$	41.43	20.76
Ptd_2Gro $(C18:2)_2(C18:1)_2$	21.69	28.39
Ptd_2Gro $(18:2)_3(20:4)$	5.22	1.32
Ptd_2Gro $(18:2)_2(18:1)(20:4)$	2.03	1.31

Data represent the mean $n = 3$

Ptd_2Gro cardiolipin, *BTHS* Barth syndrome

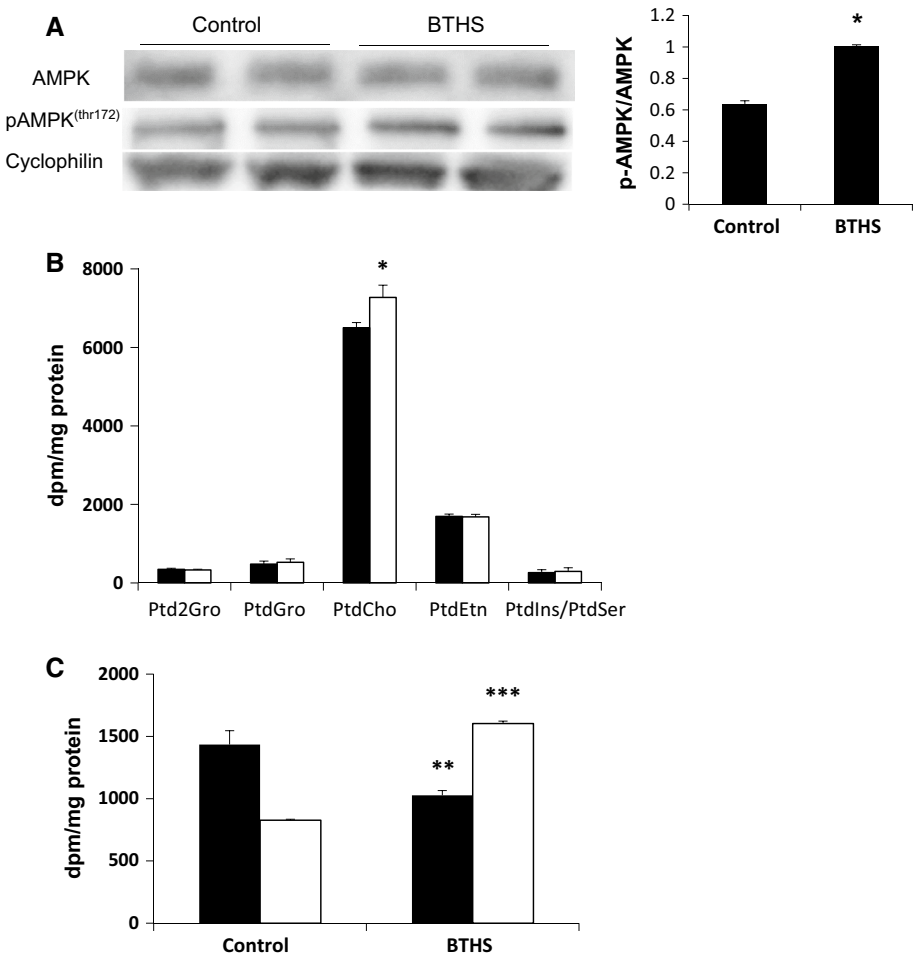

Fig. 2 Phosphorylation and expression of AMPK and D-[1-^{14}C]Glucose incorporation into glycerolipids in control and BTHS cells. **a** AMPK, pAMPK$^{(Thr172)}$ and cyclophilin expression was determined in control and BTHS lymphoblasts. *$p < 0.001$, $n = 3$. Representative Western blots are depicted. **b**, **c** D-[U-^{14}C]glucose incorporation into glycerolipids was determined in control and BTHS lymphoblasts. **b** Phospholipids: Ptd$_2$Gro, cardiolipin; PtdGro, phosphatidylglycerol; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PtdIns/PtdSer, phosphatidylinositol/phosphatidylserine. Control, *solid bars*; BTHS, *open bars*. *$p < 0.034$, $n = 3$. **c** Neutral lipids. *Solid bars*, DAG; *open bars*, TAG. **$p < 0.012$, ***$p < 0.001$, $n = 3$

to maintain cellular energy homeostasis in the setting of dysfunctional mitochondria.

A previous study had shown that *de novo* synthesis of Ptd$_2$Gro was unaltered in BTHS fibroblasts [18]. In support of this, incorporation of D-[U-^{14}C]glucose into most glycerophospholipids including Ptd$_2$Gro was unaltered in BTHS lymphoblasts compared to controls after 24 h of incubation (Fig. 2B). A small but significant 12% increase ($p < 0.034$) in incorporation into phosphatidylcholine (PtdCho) was observed. Interestingly, in the hearts of tafazzin knockdown mice, accumulation of specific choline diacyl glycerophospholipid molecular species containing linoleic acid was observed [17]. In addition, metabolome profile analysis of plasma from BTHS patients revealed an increase in the PtdCho/lysoPtdCho ratio [19]. In contrast, D-[U-^{14}C]glucose incorporation into TAG was increased 90% ($p < 0.001$) and into DAG was reduced 28% ($p < 0.01$) in BTHS cells compared to controls (Fig. 2c). The increase in TAG synthesis was specific, since total D-[U-^{14}C]glucose incorporation into cells was unaltered (control, 4.18 ± 0.23 dpm × 10^5/mg protein; BTHS, 4.21 ± 0.38 dpm × 10^5/mg protein), indicating that at least a portion of glucose taken up may be preferentially channeled into TAG. In cells incubated with [1-^{14}C]acetate, the percentage of total cellular dpm of [1-^{14}C]acetate incorporation into TAG was increased 120% ($p < 0.001$) in BTHS cells compared to control cells (Fig. 3a), confirming increased TAG synthesis. In addition, DGAT-2 activity was increased 29% ($p < 0.025$) in BTHS cells versus controls, which would explain the increased TAG synthesis (Fig. 3b). If an increase in TAG synthesis is mirrored in other tissues, this may explain why BTHS patients exhibit a higher fat mass percentage than controls [5].

Caution should be used in interpreting our observations. The lymphoblasts used in our studies are transformed cells and utilize primarily glucose for their cellular energy source. This could have a differential impact on metabolic aspects that might be observed in primary cells. In addition, the presence of abundant glucose in the culture medium, combined with a minimal demand for "work", may mask the effects of Ptd$_2$Gro loss on substrate utilization, fat accumulation, or glucose disposal. We recently showed that a reduction in Ptd$_2$Gro in human brain endothelial HCMEC/D3 cells led to increased glucose uptake and glycolysis

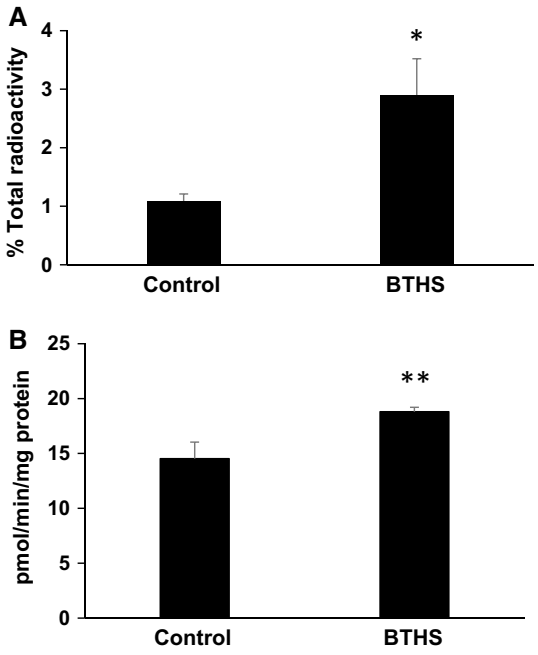

Fig. 3 Incorporation of [1-^{14}C]acetate into TAG and DGAT-2 activity in BTHS lymphoblasts. **a** Cells were incubated with [1-^{14}C]acetate, and percentage incorporation into TAG was determined in control and BTHS lymphoblasts. $^{**}p < 0.001$, $n = 3$. **b** DGAT-2 activity was determined in control and BTHS lymphoblasts. $^{**}p < 0.025$, $n = 3$

[20]. In summary, we show that BTHS lymphoblasts exhibit increased glucose uptake and TAG synthesis from glucose.

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