Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 20 (2009) 17–25
Journal of Nutritional Biochemistry

Feeding long-chain *n*−3 polyunsaturated fatty acids during gestation increases intestinal glucose absorption potentially via the acute activation of AMPK

Nicholas K. Gabler[a,b], J. Scott Radcliffe[c], Joel D. Spencer[d], Doug M. Webel[d], Michael E. Spurlock[a,b,*]

[a]*Department of Food Science and Human Nutrition, Iowa State University, Ames, IA 50011, USA*
[b]*Department of Animal Science, Iowa State University, Ames, IA 50011, USA*
[c]*Department of Animal Science, Purdue University, West Lafayette, IN 47905, USA*
[d]*JBS United Inc., Sheridan, IN 46069, USA*

Received 30 July 2007; received in revised form 19 October 2007; accepted 21 November 2007

Abstract

The current study utilized Ussing chambers to examine the impact of supplementing maternal gestation and/or lactation diets with *n*−3 polyunsaturated fatty acids (PUFA) provided via a protected fish oil (PFO) product on intestinal fatty acid profiles and ex vivo glucose uptake in the jejunum of weanling piglets. Jejunum tissues were enriched with *n*−3 PUFA as a result of feeding the sows the PFO during gestation and/or lactation ($P<.05$). Glucose uptake improved by twofold ($P<.042$) in intestinal preparations obtained from the offspring of sows fed PFO during gestation or throughout gestation/lactation versus lactation alone. This was also reflected in the jejunum protein expressions of glucose transporter 2 (GLUT2) and sodium-dependent glucose transporter 1 (SGLT1). Furthermore, adding docosahexaenoic acid (DHA) or an AMP-activated protein kinase (AMPK) agonist to the chamber buffer improved glucose uptake ($P<.05$) in intestinal preparations obtained from the offspring fed the control diet, devoid of the PFO product and containing minimal concentrations of *n*−3 PUFA. Collectively, these data indicate two important points. First, long-term exposure to *n*−3 PUFA via the maternal gestation diet effectively enhances glucose uptake in the weanling piglet, and the underlying mechanism may be associated with changes in the intestinal fatty acid profile. Secondly, there is an apparent direct and acute effect of DHA that is achieved within a time frame that precludes substantial changes in the intestinal fatty acid profile. Additionally, both mechanisms may involve activation of AMPK. Thus, *n*−3 PUFA delivered in utero and postnatally via the maternal diet may help the offspring adapt quickly to rapidly changing diets early in life and allow optimal nutrient uptake.

Keywords: *n*−3 polyunsaturated fatty acid; Glucose transporter 2; Sodium-dependent glucose transporter 1; AMP-activated protein kinase; Pig; Small intestine

1. Introduction

Glucose absorption in the jejunum of the pig occurs via the apical sodium-dependent glucose transporter 1 (SGLT1) and the basolateral glucose transporter 2 (GLUT2), both of which are regulated by a number of factors. One group of factors that our laboratory has been interested in comprises long-chain *n*−3 polyunsaturated fatty acids (PUFA). These dietary *n*−3 PUFA have been shown to normalize intestinal glucose absorption in rat pups from mothers fed diets that are low in unsaturated fatty acids [1]. However, the mechanism by which *n*−3 PUFA may augment intestinal glucose uptake is not fully understood [2].

Recent studies have suggested that the regulatory mechanism of glucose transport is also linked to the activation of AMP-activated protein kinase (AMPK), a major sensor of energy status within the cell [3]. Interestingly, AMPK is up-regulated by dietary *n*−3 PUFA in rats [4], and activation of this enzyme up-regulates GLUT2 in mice [3]. Thus, this relationship between *n*−3 PUFA and AMPK is intriguing, as we have shown recently in pigs that continuous exposure in utero and postnatally to *n*−3 PUFA

* Corresponding author. Iowa State University, Ames, IA 50011, USA.
E-mail address: mspurloc@iastate.edu (M.E. Spurlock).

0955-2863/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2007.11.009

Table 1
Gestation and lactation diets (as-fed basis)

	Gestation		Lactation	
	CONT	PFO	CONT	PFO
Ingredient (%)				
Corn	75.69	75.69	64.96	64.96
Soybean meal (48%)	18.66	18.66	27.74	27.74
Vitamin/mineral/phytase premix	4.65	4.65	6.30	6.30
Corn starch	1.00	–	1.00	–
PFO [a]	–	1.00	–	1.00
Total	100	100	100	100
Calculated nutrient content (%)				
Crude fat	3.56	3.78	3.45	3.66
Crude protein	15.17	15.26	19.09	19.18
Lysine	0.75	0.75	1.10	1.10
Phosphorus	0.77	0.77	0.81	0.81
Calcium	0.88	0.88	0.91	0.91
EPA [b]	–	0.007	–	0.007
DHA [b]	–	0.007	–	0.007
12:0, 14:0 and 16:0 [b]	–	0.013	–	0.013
Total *n*−6 fatty acids	1.58	1.58	1.43	1.43
Total *n*−3 fatty acids	0.06	0.13	0.07	0.14
n−6:*n*−3 fatty acid ratio	26.70	12.04	20.51	10.11

[a] PFO was supplied by JBS United Inc.
[b] Calculated percentage of total fat in the diet.

via the maternal gestation and lactation diets improves ex vivo glucose uptake by the jejunum after a simulated weaning stress [2]. This improvement was associated with increased proportions of *n*−3 fatty acids in the jejunal tissue, together with greater AMPK activity and increased SGLT1 and GLUT2 abundance.

Collectively, the studies summarized above indicate a clear potential to influence glucose absorption in the offspring via the maternal dietary fatty acid profile. In the present study, we sought to determine whether the efficacy of dietary *n*−3 fatty acids as modulators of glucose transport in the weanling pig varies with respect to gestation versus lactation supplementation of the maternal diet. Additionally, we tested the hypothesis that ex vivo treatment of jejunal tissue with the AMPK agonist 5-aminoimidazole-4-carboxamide riboside (AICAR) or with the *n*−3 PUFA docosahexaenoic acid (DHA) would improve ex vivo glucose uptake. The data presented herein indicate that long-chain *n*−3 PUFA supplementation of maternal diets during gestation, rather than during lactation, increases intestinal glucose absorption at weaning via increased GLUT2 translocation and SGLT1 expression, and that AMPK is a key mediator of these events in the jejunum.

2. Methods

2.1. Animals and experimental design

All animal experiments were conducted at the research facilities of JBS United Inc. in Frankfort, IN, and all procedures were approved by the JBS United Inc. Animal Care and Use Committee as per corporate policy. Thirty-two female pigs (Ausgene Line 20 dams×SPI sire) were fed one of two experimental diets for approximately 150 days to encompass the entire gestation period or 17–19 days over the lactation period before weaning. The dietary treatments (Table 1) consisted of the following: (a) basal corn/soybean meal control (no added fat; CONT); (b) the basal diet supplemented with a protected fish oil (PFO) product (Gromega 365; JBS United Inc., Sheridan, IN). The PFO product contains 21% of total fat as *n*−3 PUFA, with approximately 13% as eicosapentaenoic acid (EPA) and 13% as DHA. The gestation and lactation diet formulations have been reported previously [2] and have been formulated to meet or exceed all the requirements for gestating and lactating sows [5]. Sows and piglets had access to water at all times.

On farrowing after approximately 150 days on the gestation diets, litters were standardized to 10 piglets per sow. To accomplish exposing the piglets to the *n*−3 fatty acids only during the gestation or suckling (lactation) period, litters were reciprocally switched such that dams fed the CONT diet received piglets from a dam fed the PFO diet and vice versa for 15–19 days. The four treatments now consisted of gestation/lactation feeding to give CONT/CONT, CONT/PFO, PFO/PFO or PFO/CONT piglets. Thereafter, at 15–19 days of age, one medium-sized piglet (5.4±0.50 kg) per litter was randomly transferred to a separate room from the dams, group penned and fasted overnight to simulate the weaning process (total *n*=6 per treatment). On the following morning, piglets were euthanized, and tissue samples were collected. Small intestinal jejunum and muscle samples were collected and frozen in liquid nitrogen, and additional jejunum samples were placed in 10% formalin for later analysis.

2.2. Fatty acid analysis

Lipids were extracted from piglet muscle and liver by the method of Lepage and Roy [6], with minor modifications. Briefly, 0.5 g of tissue was homogenized in 2.5 ml of 4:1 methanol:hexane, and then 200 μl of a 3.7-mmol heptadecanoic acid/L methanol solution was added to each sample as an internal standard. Fatty acid methyl esters were analyzed by gas chromatography on Hewlett-Packard model 6890 (Hewlett-Packard, Palo Alto, CA) fitted with an Omegawax 320 (30 m×0.32 mm ID; 0.25 μm) capillary column (Sigma-Aldrich, St. Louis, MO). Hydrogen was the carrier gas. The temperature program ranged from 80°C to 250°C, with a temperature rise of 5°C/min. The injector and detector temperatures were 250°C, and 1 μl of the sample was injected and run splitless. Fatty acids were identified by their retention times on the column as judged from appropriate standards.

2.3. Ussing chamber

Proximal jejunum samples, starting 40 cm from the stomach and consisting of a 20- to 30-cm segment of the

jejunum, were removed and placed in chilled Krebs–Henseleit buffer (pH 7.4), which consisted of the following: 25 mM $NaHCO_3$, 120 mM NaCl, 1 mM $MgSO_4$, 6.3 mM KCl, 2 mM CaCl and 0.32 mM NaH_2PO_4. The tissue was aerated continuously until clamped in the Ussing chambers in the laboratory. Tunica muscularis was removed from two jejunal segments per pig and mounted immediately in Ussing chambers (DVC 1000; World Precision Instruments, New Haven, CT). Each segment was bathed on its mucosal and serosal surfaces (opening area, 1.0 cm^2) with 8 ml of Krebs solution and gassed with 95% O_2–5% CO_2 mixture. Voltage was clamped at 0 mV by an external current after correction for solution resistance. After a 30-min period to allow the tissues to stabilize, they were challenged with 10 mM D-glucose added to serosal buffer and an equimolar concentration of mannitol added to the mucosal buffer. Additionally, to test a mechanistic hypothesis, jejunum samples from some CONT/CONT piglets were mounted, stabilized and treated (mucosal) with 0.1 mM DHA or 2.5 mM AICAR solubilized in 20 mM taurocholic acid (bile salt). Glucose uptake was then assessed after 20 min, with the tissues challenged with 10 mM D-glucose as described earlier. The potential difference across the tissue was measured for 30 min after each challenge by open-circuit conditions every 10 s due to a short-circuit current being delivered by a voltage clamp apparatus. The change in maximal current was recorded, and tissue conductance was calculated from the short-circuit current and potential difference using Ohm's law. This procedure was repeated on four different days with a pig from each dietary regimen to achieve a total of four pigs per treatment.

2.4. Immunoblot analysis of glucose transport proteins in total and brush border membrane (BBM) preparations

Fresh intact proximal jejunum was removed, washed with saline and placed on ice, while approximately 4 g of mucosa was removed and transferred to cold 2 mM Tris–HCl buffer (pH 7.1) containing 50 mM mannitol and protease inhibitors (5 μM aprotinin, leupeptin and pepstatin). The mucosa was then homogenized, and PEG 4000 was added to a final concentration of 10% and stirred on ice for 15 min. The homogenate was then centrifuged for 15 min at 7500×*g*, and the resulting supernatant fraction was centrifuged at 27,000×*g* for 60 min at 4°C. The pellet was washed in suspension buffer (10 mM Tris–HCl, pH 7.1, containing 300 mM mannitol and 5 μM protease inhibitors aprotinin, leupeptin and pepstatin) and collected again by centrifugation for 5 min at 27,000×*g* and 4°C. The crude BBM pellet was suspended in 1 ml of suspension buffer. For preparation of total membranes, frozen jejunum sections (1 g) were homogenized on ice in 700 μl of Buffer A (50 mM Tris–HCl pH 7.5, 50 mM NaF, 5 mM sodium pyrophosphate, 1 mM EDTA, 1 mM DTT, 0.1 mM phenylmethylsulfonyl fluoride and 10% glycerol) containing 1% Triton X-100 and 5 μM aprotinin, leupeptin and pepstatin. The homogenates were centrifuged at 6000×*g* for 20 min at 4°C to remove insoluble materials. The protein concentrations of the total and BBM preparations were determined using BCA reagents (Pierce, Rockford, IL). The final total and BBM preparations were frozen at −80°C until assayed. The purity of the BBM preparations as measured by alkaline phosphatase was not affected by treatment (data not shown).

The abundance of GLUT2 and SGLT1 proteins in total and crude BBM was determined by Western blot analysis. Briefly, membrane preparations containing 250 μg of protein were immunoprecipitated at room temperature for 2 h using the Catch and Release v2.0 Reversible Immunoprecipitation System (Millipore, Billerica, VA). Both GLUT2 and SGLT1 were immunoprecipitated with 1:100 primary antibody (Chemicon International, Temecula, CA) dilution. Immunoprecipitated proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using a 12% resolving gel, transferred to a nitrocellulose membrane and incubated overnight with primary GLUT2 or SGLT1 antibody (1:1000 dilution).

Whole jejunal protein lysates containing 250 μg of protein were prepared using Buffer A as described previously and immunoprecipitated at room temperature for 2 h using the Catch and Release v2.0 Reversible Immunoprecipitation System (Millipore). Both phospho-AMPKα Thr172 and total AMPKα were immunoprecipitated with 1:100 primary antibody dilution in a total volume of 250 μl. Protein complexes were eluted from the column using 40 μl of 1× denaturing elution buffer in accordance with the manufacturer's directions. Total eluent protein was separated by SDS-PAGE using a 12% resolving gel. Proteins were transferred to a nitrocellulose membrane and probed overnight with primary antibody for total or phospho-AMPKα at a dilution of 1:1000.

After the overnight primary antibody treatments, all membranes were incubated with a 1:20,000 dilution of goat–anti-rabbit IgG complexed to horseradish peroxidase (Pierce) for 1 h at room temperature. Blots were developed using the SuperSignal West Pico Chemiluminescent Substrate System (Pierce) and exposed to film. Image analysis was performed using the Quantity One 1-D analysis software (Bio-Rad, Hercules, CA).

2.5. Statistical analyses

All data are expressed as mean±S.E.M. The effects of dietary treatment regimen were determined by the PROC MIXED procedure in SAS (Version 9.1; SAS Institute, Cary, NC), and treatment differences were established using the least significant differences procedure when protected by a significant *F*-value. The effect of gestation, lactation or gestation/lactation on *n*−3 PUFA feeding was assessed in the model. Litter/piglet was considered the experimental unit, and experimental replicate or day of harvest was considered a random effect. Differences were deemed significant at $P<.05$, and tendencies were noted at $P<.10$.

3. Results

3.1. Piglet tissues reflect dietary fatty acid profiles of maternal diet

Fatty acid profiles for the jejunum and longissimus dorsi muscle are presented in Tables 2 and 3, respectively. Feeding PFO throughout gestation and lactation resulted in significantly ($P<.05$) higher $n-3$ PUFA contents in both the jejunum and the muscle versus the CONT/CONT regimen. This increase was achieved by both DHA and EPA in the jejunum, but the muscle showed enrichment largely as DHA. Discontinuing the PFO diet at the onset of lactation caused a significant decrease in the DHA, EPA and total $n-3$ contents in both tissues. However, feeding the PFO diet for the lactation period alone achieved similar enrichment as did feeding this $n-3$ source for the entire 150 days.

3.2. Glucose transport

We compared changes in active glucose transport in the jejunum of piglets weaned from dams fed the CONT diet or the PFO diet. Feeding PFO throughout gestation and lactation increased glucose uptake by 500% (5 vs. 25 μA/cm^2; $P<.05$; Fig. 1), and provision of the $n-3$ source in gestation alone improved glucose uptake by about 400%. In contrast, feeding PFO in lactation only precluded any significant enhancement in glucose uptake (15 vs. 5 μA/cm^2, respectively; $P=.16$).

Table 2
Fatty acid composition of jejunum samples obtained from piglets weaned from dams fed the control (CONT) and PFO dietary regimens during gestation and/or lactation

Fatty acid	CONT/CONT (g/100 g)	CONT/PFO (g/100 g)	PFO/PFO (g/100 g)	PFO/CONT (g/100 g)
14:0	0.06	0.09	0.05	0.10
16:0	19.82	19.31	20.13	21.52
16:1	1.45	1.27	1.24	1.17
18:0	22.14	25.86	23.28	20.03
18:1	13.43	12.31	11.64	14.60
18:2($n-6$)	21.65	20.28	20.25	20.05
18:3($n-6$)	0.25	0.16	0.24	0.24
18:3($n-3$)	0.32	0.33	0.34	0.37
20:2	0.42	0.24	0.22	0.40
20:3($n-6$)	0.63	0.46	0.69	0.51
20:4($n-6$)	14.66	12.29	13.61	14.75
20:5($n-3$)	0.18^a	0.73^b	0.74^b	0.25^a
22:4	1.82	1.27	1.17	1.94
22:5($n-3$)	1.11^a	1.33^b	1.38^b	1.40^b
22:6($n-3$)	0.27^a	3.68^c	4.51^c	2.15^b
Other	1.77	0.39	0.51	0.54
Total	100.00	100.00	100.00	100.00
Saturated	42.69	45.64	43.88	42.19
Total $n-3$	2.88^a	6.06^c	6.97^c	4.17^b
Total $n-6$	37.20	33.20	34.79	35.54
$n-6/n-3$	12.91^b	5.47^a	5.16^a	8.81^a

Results are presented as the means of four piglets per treatment.
Within rows, means without a common letter differ ($P<.05$).

Table 3
Fatty acid composition of the longissimus dorsi muscle obtained from piglets of dams weaned from dams fed the CONT and PFO dietary regimens during gestation and/or lactation

Fatty acid	CONT/CONT (g/100 g fatty acid)	CONT/PFO (g/100 g fatty acid)	PFO/PFO (g/100 g fatty acid)	PFO/CONT (g/100 g fatty acid)
14:0	0.25	0.22	0.16	0.51
16:0	21.41	21.23	20.64	20.48
16:1	2.68	2.55	2.44	3.43
18:0	15.77	15.22	14.59	15.82
18:1	13.51	12.92	14.94	17.79
18:2($n-6$)	26.92	25.45	23.76	23.46
18:3($n-6$)	0.00	0.00	0.08	0.00
18:3($n-3$)	0.39	0.36	0.32	0.39
20:2	0.61	0.59	0.63	0.68
20:3($n-6$)	1.05	1.09	0.93	1.02
20:4($n-6$)	13.32	12.20	12.43	12.18
20:5($n-3$)	0.34^a	0.98^b	3.29^c	0.30^a
22:4	2.14	1.55	1.48	1.71
22:5($n-3$)	1.52	1.87	1.83	1.44
22:6($n-3$)	0.00^a	1.97^c	2.48^c	0.70^b
Other	0.09	0.00	0.00	0.10
Total	100.00	100.00	100.00	100.07
Saturated	37.43	38.47	35.39	36.81
Total $n-3$	2.25^a	5.18^{bc}	7.92^c	2.84^{a,b}
Total $n-6$	41.29	38.74	37.20	36.65
$n-6/n-3$	18.64^a	7.55^b	5.89^b	14.42^a

Results are presented as the means of four piglets per treatment.
Within rows, means without a common letter differ ($P<.05$).

3.3. Expression of glucose transport proteins in the jejunum

Immunoblots for GLUT2 abundance in crude BBM preparations showed a small but significant ($P<.05$) enrichment in the latter fraction obtained from piglets of dams consuming the PFO diets (Fig. 2A), but there was no apparent change in abundance in total homogenates (Fig. 2B). This result was not influenced by the duration of the PFO regimen, nor was it specific to the gestation or lactation period.

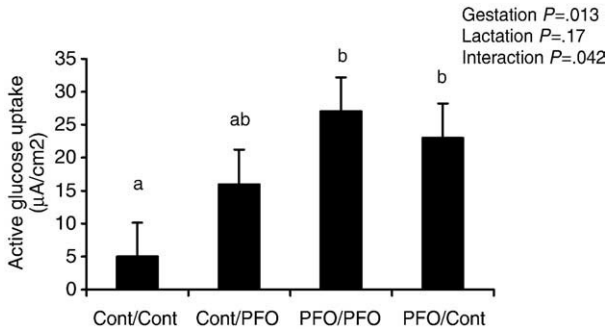

Fig. 1. Ex vivo active glucose uptake by the proximal jejunum of piglets at 21 days of age after feed deprivation for 24 h to simulate weaning stress. Dams were fed the control (CONT) and PFO dietary regimens during gestation and/or lactation (G/L). Data represent the means of six piglets per treatment. Means without a common letter are significantly different ($P<.05$).

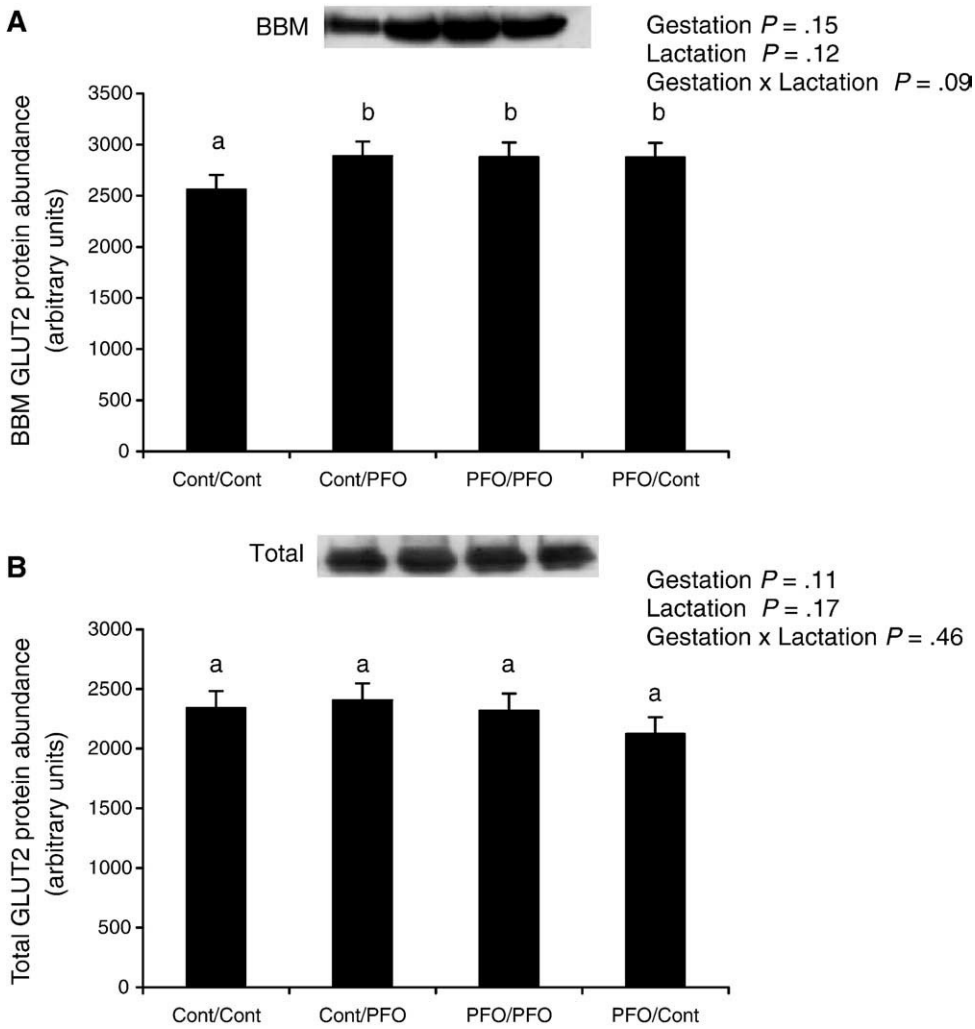

Fig. 2. Abundance of GLUT2 protein in (A) crude BBMs and (B) total tissue preparations from the proximal jejunum of piglets at 21 days of age after feed deprivation for 24 h to simulate weaning stress. Dams were fed the control (CONT) and PFO dietary regimens during gestation and/or lactation (G/L). Data represent the mean±S.E. of six piglets per treatment. Means without a common letter are significantly different ($P<.05$).

We performed similar immunoblots for SGLT1. As seen in Fig. 3A, the CONT/PFO dietary regimen tended to increase the abundance of the SGLT1 protein in the BBM preparations, but only the PFO/CONT regimen was significant ($P<.05$). In contrast, feeding PFO in any dietary regimen increased ($P<.06$) the abundance of SGLT1 protein in the total homogenate (Fig. 3B).

3.4. n−3 PUFA activates AMPK in the jejunum

Next, we assessed the ability of $n-3$ PUFA to activate AMPK, as indicated based on semiquantitative Western blot analyses for the total and phosphorylated (Thr172) AMPK proteins in jejunum homogenates. Either the CONT/PFO or the PFO/PFO dietary regimen resulted in an approximate 20% increase ($P<.05$) in phospho-AMPK versus the CONT/CONT regimen (Fig. 4). Similarly, gestational feeding of PFO (i.e., PFO/CONT) resulted in an approximate 10% increase ($P<.05$). The abundance of total AMPK protein was unchanged.

3.5. Effect of AICAR and DHA on glucose transport

To establish whether $n-3$ PUFA or activation of AMPK directly regulates glucose uptake, CONT/CONT jejunum sections were mounted in Ussing chambers, and the mucosal side was exposed to 0.1 mM DHA or 2.5 mM AICAR. Both DHA and AICAR resulted in an approximate 50% increase ($P<.05$) in glucose transport versus the vehicle (Fig. 5).

4. Discussion

It is well established that the maternal diet during gestation and lactation influences fetal and postnatal growth and development, and regulates intestinal function [7]. The fetus obtains most of its energy requirements through the oxidation of glucose, lactate and amino acids, while during suckling, the newborn oxidizes a high-fat low-carbohydrate diet (milk) for energy [8]. This highlights the need for mammalian species to undergo profound structural and functional

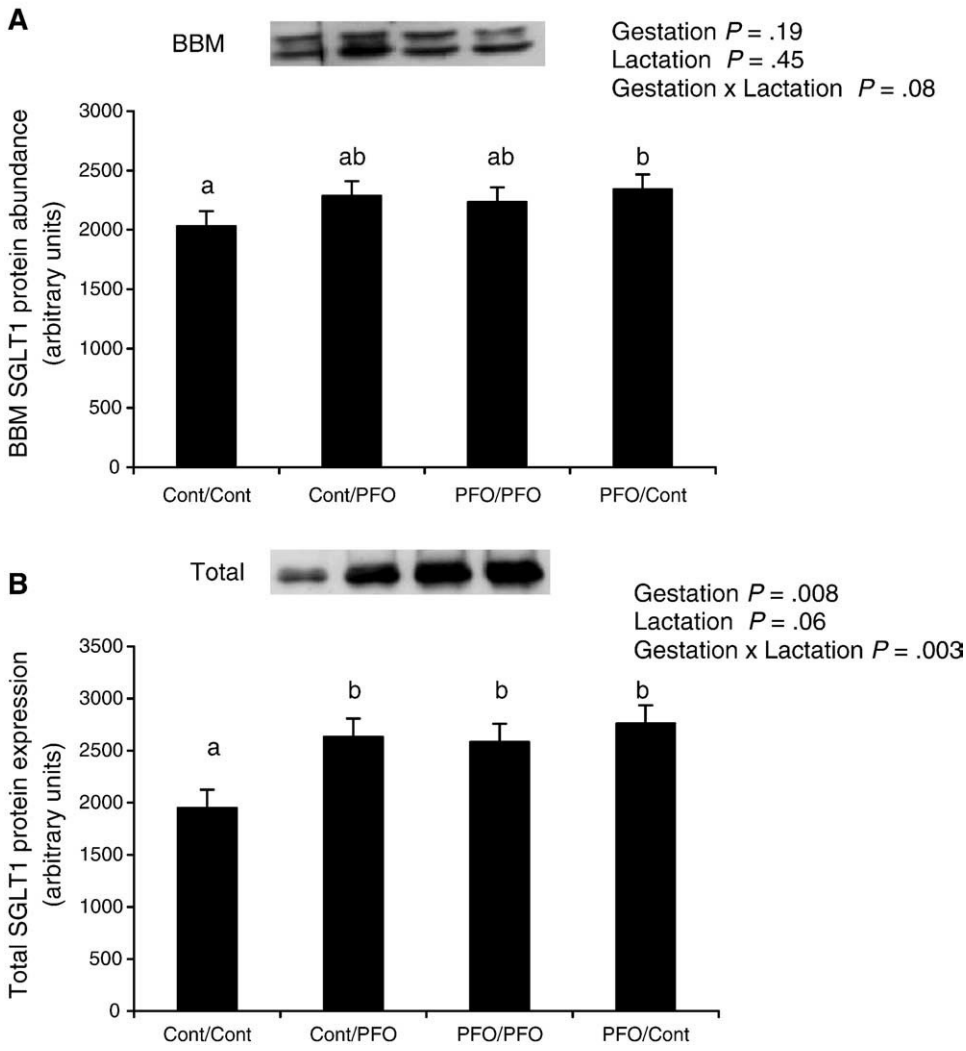

Fig. 3. Abundance of SGLT1 protein in (A) crude BBMs and (B) total tissue preparations from the proximal jejunum of piglets at 21 days of age after feed deprivation for 24 h to simulate weaning stress Dams were fed the control (CONT) and PFO dietary regimens during gestation and/or lactation (G/L). Data represent the mean±S.E. of six piglets per treatment. Means without a common letter are significantly different ($P<.05$), and CONT/CONT and CONT/PFO differed at $P<.10$.

changes in their small intestine to accommodate the changes in nutrition [8,9]. Previously, we have reported that feeding an $n-3$ PUFA source throughout gestation and lactation increases ex vivo glucose uptake in the proximal jejunum of piglets subjected to a simulated weaning process [2].

The data presented herein extend those findings and establish several key points with respect to maternal provision of $n-3$ fatty acids as a means of influencing tissue concentrations in the offspring. First of all, intestinal loading with $n-3$ fatty acids can be achieved quite quickly in the offspring, as feeding the $n-3$ PUFA source (i.e., PFO) during lactation alone (17–21 days) was as effective as feeding during gestation alone or during the entire 150-day gestation/lactation duration. It is also intriguing to note that, whereas removing the PFO from the lactation diet partially depleted the intestine of accumulated DHA, no such depletion of EPA occurred. Although the mechanisms underlying this difference are not yet apparent, the metabolism and utilization of DHA result in a much more rapid turnover rate than the metabolism and utilization of EPA. Secondly, although muscle was not a focus of this study, we sampled this tissue for comparative purposes. As with the intestine, the DHA content of muscle reflected the maternal diet, with the level of enrichment declining sharply unless the source was included in the lactation diet. However, in contrast with the gut, adding PFO to the maternal diets did not significantly enrich the muscle of the offspring with EPA. It is logical to conclude that either the muscle preferentially utilized the EPA or muscle accumulation of this $n-3$ fatty acid was limited. It is perhaps important to note that without $n-3$ supplementation of the maternal diets, muscle concentrations of EPA were not detected.

Interestingly, some of the differences in fatty acid incorporation and gut function in the offspring may be

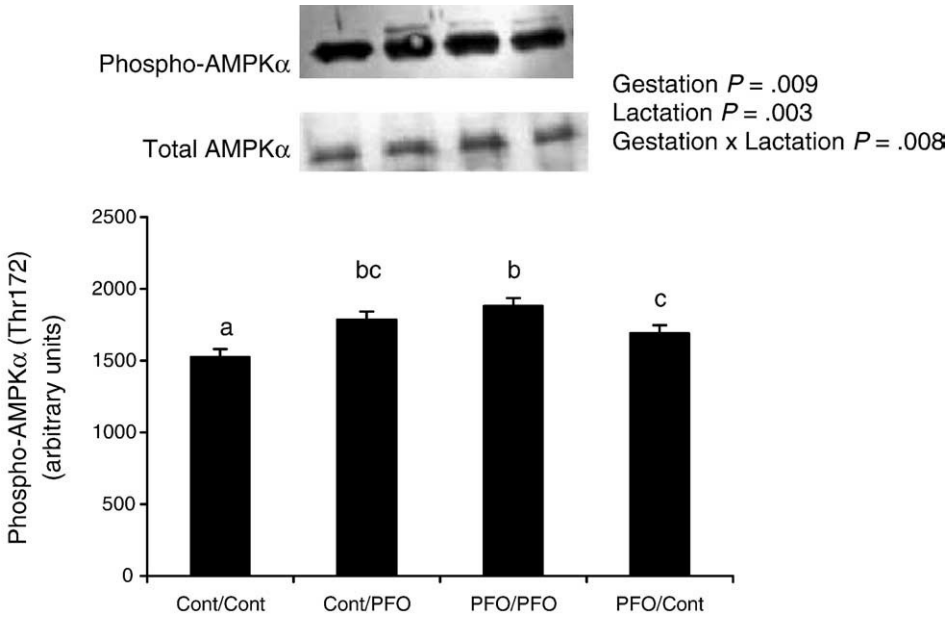

Fig. 4. Protein abundance of phospho-AMPKá (Thr172) protein in total tissue extracts from the proximal jejunum of piglets at 21 days of age after feed deprivation for 24 h to simulate weaning stress. Dams were fed the control (CONT) and PFO dietary regimens during gestation and/or lactation (G/L). Data represent the mean±S.E. of four piglets per treatment. Means without a common letter are significantly different ($P<.05$).

explained by in utero swallowing of amniotic fluid. The maternal–fetal pregnancy environment can be a dramatic influence on the imprinting of regulatory mechanisms controlling ingestion behavior in species in which swallowing behavior develops in utero [10]. Furthermore, recent research in rats has shown that maternal dietary fat, especially the balance of $n-6$ and $n-3$ fatty acids, can influence the fetal intestinal membrane fatty acid composition via manipulation of amniotic fluid and consequential swallowing by the fetus and placental transport [11].

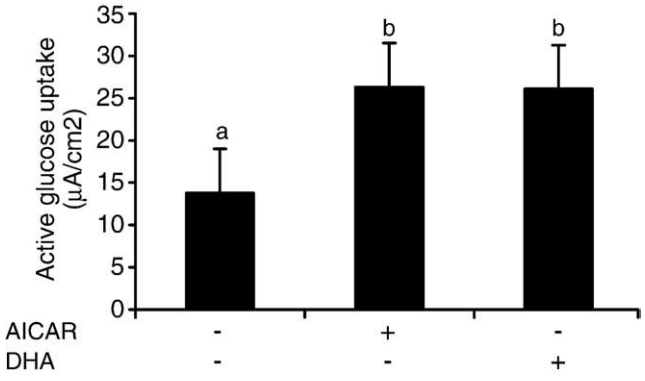

Fig. 5. The effects of DHA and activation of AMPK on ex vivo active glucose uptake by piglet jejunum. Jejunum sections were obtained from piglets at 21 days of age after feed deprivation for 24 h to simulate weaning stress. Only piglets fed the CONT/CONT dietary regimen were used. After mounting in Ussing chambers, the sections were stabilized, and the mucosal side was stimulated with 2.5 mM AICAR to activate AMPK, or 0.1 mM DHA, for 20 min prior to the addition of glucose (10 mM). Data represent the mean±S.E. of five independent experiments (i.e., five individual piglets). Means without a common letter are significantly different ($P<.05$).

As regards to the ex vivo glucose uptake, incorporating the $n-3$ PUFA source into gestation or gestation/lactation diets increased uptake by some threefold to fourfold. However, inclusion in the lactation diet alone was insufficient to alter transport. This seems paradoxical given the fact that lactation exposure alone did maximize intestinal $n-3$ content and that gestational exposure alone resulted in marked depletion of DHA to a concentration approximately half that of the CONT/PFO regimen. This possibly indicates that there is a determinant of transport that is responsive to long-term $n-3$ exposure and that the 21-day lactation exposure is insufficient to achieve this response.

We investigated intestinal BBM glucose transporters (GLUT2 and SGLT1) as potential explanations of this phenomenon. GLUT2 increased with any exposure to PFO, and SGLT1 was significantly greater only in pigs from dams fed the PFO/CONT regimen. Thus, the abundance of these transporters in the BBM was not a sole explanation for the effects of maternal $n-3$ supplementation on ex vivo glucose uptake. Consequently, we also quantified AMPK and its phosphorylated (activated) form. Maternal supplementation via either the gestation diet or the lactation diet was sufficient to maximize AMPKα phosphorylation, whereas continual supplementation (i.e., PFO/PFO) was equally effective, but not better. Thus, the abundance of phopsho-AMPK was maximally increased by the same dietary regimen that failed to significantly up-regulate ex vivo glucose transport.

Collectively, our findings indicate that supplementation of the maternal diet with $n-3$ fatty acids as a means of delivery to the offspring, rather than simple abundance, may influence the functionality of glucose transporters in the jejunum and that activation of AMPK is not a

prerequisite. Whatever the mechanism, given the fact that the CONT/PFO maternal regimen did not significantly increase glucose uptake, we were somewhat surprised to see that short-term exposure to DHA in the Ussing chamber resulted in an approximate 70% increase in active glucose uptake. This finding clearly indicates the potential for an acute regulation of glucose uptake by DHA. Because activation of AMPK with AICAR achieved a similar response and because of the indications that *n*−3 PUFA activate AMPK in other tissues [4], we cautiously speculate that the acute effect of DHA was mediated by AMPK. However, further studies need to be carried out to allude to the exact mechanism of action of DHA on AMPK. Inhibition of AMPK with adenine 9-β-D arabinofuranoside or siRNA could be used to see whether this blocks the DHA effect on AMPK activation.

The activation of AMPK has resulted in enhanced glucose uptake by increasing the levels of GLUT2 in the BBMs of mice jejunum [3]. Since dietary *n*−3 PUFA enhance rat hepatic AMPK activity in vivo [4], we can cautiously speculate that it is the DHA or long-chain *n*−3 PUFA that may be acutely signaling glucose transporter proteins through AMPK. However, unlike Walker et al. [3], who reported that AICAR decreased the levels of cellular SGLT1 in mice and had no effect on mRNA levels, in the current study, we observed an increase in the total SGLT1 protein expression due to PFO feeding. This suggests that SGLT1 is not regulated through an AMPK-dependent mechanism like GLUT2. However, confirmation of our current results will require additional experimentation.

Another plausible explanation for the paradoxical results observed in the ex vivo glucose uptake data is the kinetics of fatty acid replacement and incorporation into different membrane compartments or subcellular fractionation. This subcellular distribution of PUFA has received minimal attention in the literature to date. However, differences in the fatty acid composition of human kidney nuclei, cytosol, plasma membranes and mitochondria subcellular fractions have been shown to vary in conjugated linoleic acid content [12]. Furthermore, rats fed corn oil diets versus fish oil diets had differential subcellular (nuclear matrix vs. membrane) *n*−3 and *n*−6 PUFA compositions [13]. Differences have also been reported in rats that are dependent on age and, to some extent, the dietary *n*−6/*n*−3 PUFA ratio, regarding the kinetics of fatty acid incorporation and replacement in specific phospholipid fractions and membrane compartments [14]. Taken together, these data suggest that different fatty acid contents in the membrane and organelles of cells exist, and that one could cautiously speculate that these differences could alter the functionality of membrane and intracellular transporters such as SGLT1 and GLUT2.

In support of our current study, continuously feeding rat dams a low *n*−6:*n*−3 diet during pregnancy and lactation has been associated with a high glucose transport rate in the small intestine [7]. However, switching dams from a high or low *n*−6:*n*−3 content diet during gestation to a high-DHA diet (lactation) had little effect on D-glucose uptake in the rat pup jejunum samples, although, unlike the current study where the sows were on the gestation diet prior to conception, Jarocka-Cyrta et al. [7] only had their rat dams on the gestation diets for 2 weeks prior to switching. Additionally, only amniotic fluid fatty acid composition was measured, and it was unclear if the maternal diets (gestation and/or lactation) enabled sufficient enrichment of the offspring's tissue. It has also been shown that fetal swallowing is important in small intestine development and, thus, amniotic fluid may be a potential source of fetal nutrition; this highlights the importance of the gestational nutrition of the mother [15,16]. Additionally, Jarocka-Cyrta et al. [7] did not report whether diet-associated changes influenced the abundance and localization of the glucose transporters required for glucose uptake in the rat. Nonetheless, the mother's diet during gestation is important for normal fetal growth, and dietary fatty acids can influence glucose uptake in the small intestine.

In summary, our investigation demonstrates that on weaning, piglet jejunum glucose flux is up-regulated if it is exposed to *n*−3 PUFA, particularly during gestation and gestation/lactation. The increase in glucose uptake appears to be orchestrated by two separate mechanisms: first, increased total jejunum SGLT1 protein content due to PFO feeding; and, second, in an acute manner, potentially through the *n*−3 PUFA activation of jejunum AMPK resulting in the increased translocation of GLUT2 to the BBM. Thus, it is feasible to modulate small intestinal transport function via in utero and postnatal manipulation of the dams' diet. This, in turn, may help the offspring adapt quickly to rapidly changing diets early in life and allow optimal nutrient uptake.

Acknowledgments

The authors thank Neil Jackson, Eric Parr and the staff of JBS United Inc.'s T.C. Bache farm for caring for the pigs and for assisting in sample collection. Additional thanks goes to Dr. Kari Saddoris and students Blair Aldridge and Lindsay Sims at Purdue University (who work for Dr. Radcliffe) for their expertise and help with the Ussing chambers component of this paper. This work was funded, in part, by JBS United Inc. Additionally, Drs. Spencer and Webel are employed by JBS United Inc.; however, the other authors declare no conflicts of interest.

References

[1] Vicario M, Pedragosa E, Rivero M, Moreto M, Amat C. Dietary unsaturated long-chain fatty acids modify D-glucose absorption in weaning rats. J Pediatr Gastroenterol Nutr 2005;40:151–6.

[2] Gabler NK, Spencer JD, Webel DM, Spurlock ME. In utero and postnatal exposure to long chain (*n*−3) PUFA enhances intestinal glucose absorption and energy stores in weanling pigs. J Nutr 2007; 137:2351–8.

[3] Walker J, Jijon HB, Diaz H, Salehi P, Churchill T, Madsen KL. 5-Aminoimidazole-4-carboxamide riboside (AICAR) enhances GLUT2-dependent jejunal glucose transport: a possible role for AMPK. Biochem J 2005;385:485–91.
[4] Suchankova G, Tekle M, Saha AK, Ruderman NB, Clarke SD, Gettys TW. Dietary polyunsaturated fatty acids enhance hepatic AMP-activated protein kinase activity in rats. Biochem Biophys Res Commun 2005;326:851–8.
[5] NRC. Nutrient requirements of swine. 10th ed. Washington (DC): National Academic Press; 1998.
[6] Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. J Lipid Res 1986;27:114–20.
[7] Jarocka-Cyrta E, Perin N, Keelan M, Wierzbicki E, Wierzbicki T, Clandinin MT, et al. Early dietary experience influences ontogeny of intestine in response to dietary lipid changes in later life. Am J Physiol 1998;275:G250–8.
[8] Ferre P, Decaux JF, Issad T, Girard J. Changes in energy metabolism during the suckling and weaning period in the newborn. Reprod Nutr Dev 1986;26:619–31.
[9] Girard J, Ferre P, Pegorier JP, Duee PH. Adaptations of glucose and fatty acid metabolism during perinatal period and suckling–weaning transition. Physiol Rev 1992;72:507–62.
[10] Ross MG, Nijland MJ. Fetal swallowing: relation to amniotic fluid regulation. Clin Obstet Gynecol 1997;40:352–65.
[11] Friesen R, Innis SM. Maternal dietary fat alters amniotic fluid and fetal intestinal membrane essential *n*−6 and *n*−3 fatty acids in the rat. Am J Physiol Gastrointest Liver Physiol 2006;290: G505–10.
[12] Hoffmann K, Blaudszun J, Brunken C, Hopker WW, Tauber R, Steinhart H. Distribution of polyunsaturated fatty acids including conjugated linoleic acids in total and subcellular fractions from healthy and cancerous parts of human kidneys. Lipids 2005;40:309–15.
[13] Iritani N, Fukuda H, Matsumura Y. Effects of corn oil- and fish oil-supplemented diets on phospholipid fatty acid composition of rat liver nuclei. Biochim Biophys Acta 1988;963:224–30.
[14] Abel S, Gelderblom WC, Smuts CM, Kruger M. Thresholds and kinetics of fatty acid replacement in different cellular compartments in rat liver as a function of dietary *n*−6/*n*−3 fatty acid content. Prostaglandins Leukot Essent Fatty Acids 1997;56:29–39.
[15] Mulvihill SJ, Stone MM, Debas HT, Fonkalsrud EW. The role of amniotic fluid in fetal nutrition. J Pediatr Surg 1985;20:668–72.
[16] Mulvihill SJ, Stone MM, Fonkalsrud EW, Debas HT. Trophic effect of amniotic fluid on fetal gastrointestinal development. J Surg Res 1986;40:291–6.