Enhanced glycogen metabolism in adipose tissue decreases triglyceride mobilization

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Submitted 14 December 2009; accepted in final form 26 April 2010

Markan KR, Jurczak MJ, Allison MB, Ye H, Sutanto MM, Cohen RN, Brady MJ. Enhanced glycogen metabolism in adipose tissue decreases triglyceride mobilization. Am J Physiol Endocrinol Metab 299: E117–E125, 2010. First published April 27, 2010; doi:10.1152/ajpendo.00741.2009.—Adipose tissue is a primary site for lipid storage containing trace amounts of glycogen. However, refeeding after a prolonged partial fast produces a marked transient spike in adipose glycogen, which dissipates in coordination with the initiation of lipid resynthesis. To further study the potential interplay between glycogen and lipid metabolism in adipose tissue, the aP2-PTG transgenic mouse line was utilized since it contains a 100- to 400-fold elevation of adipocyte glycogen levels that are mobilized upon fasting. To determine the fate of the released glucose 1-phosphate, a series of metabolic measurements were made. Basal and isoproterenol-stimulated lactate production in vitro was significantly increased in adipose tissue from transgenic animals. In parallel, basal and isoproterenol-induced release of nonesterified fatty acids (NEFAs) was significantly reduced in transgenic adipose tissue vs. control. Interestingly, glycerol release was unchanged between the genotypes, suggesting that enhanced triglyceride resynthesis was occurring in the transgenic tissue. Qualitatively similar results for NEFA and glycerol levels between wild-type and transgenic animals were obtained in vivo during fasting. Additionally, the physiological upregulation of the phosphoenolpyruvate carboxykinase cytosolic isoform (PEPCK-C) expression in adipose upon fasting was significantly blunted in transgenic mice. No changes in whole body metabolism were detected through indirect calorimetry. Yet weight loss following a weight gain/loss protocol was significantly impeded in the transgenic animals, indicating a further impairment in triglyceride mobilization. Cumulatively, these results support the notion that the adipocyte possesses a set point for glycogen, which is altered in response to nutritional cues, enabling the coordination of adipose glycogen turnover with lipid metabolism.

Glycogenolysis; fasting; lipolysis; protein phosphatase-1

THE RATE OF OBESITY IS GROWING on an epidemic scale (11, 21). Estimates from the World Health Organization suggest that 300 million people worldwide are obese and more than 1 billion are overweight (20, 21). Obesity is associated with a variety of comorbidities, including increased cardiovascular risk, obstructive sleep apnea, and type 2 diabetes mellitus (T2DM), collectively referred to as the metabolic syndrome (23). Insulin resistance in obesity and T2DM is attributable to numerous alterations in the handling of energy, including an impaired suppression of lipolysis and hepatic gluconeogenesis and decreased insulin-dependent glucose transport and metabolism in skeletal muscle (8).

High circulating levels of NEFA have been strongly implicated in global insulin resistance and the impairment of glucose metabolism (31) arising from an inability of the adipose tissue to safely store lipid, resulting in ectopic accumulation in other tissues, as reviewed elsewhere (5). Insulin signaling normally promotes triglyceride synthesis and storage in the central lipid droplet of adipocytes. Glucose uptake is increased via the insulin-stimulated translocation of GLUT4 facilitative transporters to the plasma membrane (25). Carbohydrate metabolism intersects with triglyceride storage through the generation of glycerol-3-phosphate (G3P) by way of dihydroxyacetone phosphate produced by glucose partially passing through glycolysis. Conversely, caloric restriction activates catecholamine signaling, which initiates the hydrolysis and mobilization of triglyceride, released as NEFA and glycerol out of adipocytes for use by other tissues.

In addition to playing a central role in global lipid metabolism, adipose tissue has also arisen as a vital endocrine tissue. An array of adipokines are secreted in response to various nutritional cues, such as adiponectin and leptin. Although mechanisms controlling the regulated biosynthesis of leptin remain unclear, its secretion is believed to occur only in response to insulin-stimulated glucose metabolism (18). Circulating levels act as a satiety signal in the brain, thereby linking adipocyte energy status with central nervous system control of feeding (7). However, the metabolic and molecular links between glucose metabolism and leptin secretion remain incompletely understood.

Glucose entering the fat cell can also be stored as glycogen (19, 24), albeit at levels far below muscle and liver. Interestingly, the link between carbohydrate and lipid metabolism in the adipocyte first arose through the study of glycogen metabolism during nutritional transition (37). It was observed in the 1940s that feeding rats after a prolonged partial fast produced a substantial transient spike in adipose glycogen that preceded lipid resynthesis. A recent study using chronic leptin administration to deplete adipose mass followed by a rapid transition to hypoalimentation recapitulated the marked glycogen levels’ oscillation prior to restoration of lipogenesis (3). It has been postulated that the modest glycogen stores in adipose tissue may provide substrate for triglyceride synthesis (33, 34). Yet the role of glycogen turnover within adipocyte metabolism during various metabolic states remains poorly defined. We have previously generated a transgenic mouse in which the protein phosphatase-1 glycogen-targeting subunit protein targeting to glycogen (PTG) was expressed under the control of the aP2 promoter (16). Glycogen levels in adipose tissue were
elevated 100- to 400-fold in the aP2-PTG mice. Furthermore, glycogen stores were significantly mobilized following an overnight fast, indicating that a new set point for glycogen metabolism had been achieved. In the current study, this transgenic model was utilized to further investigate the metabolic links between lipid and glycogen metabolism in adipose tissue and provide evidence that glycogen mobilization can contribute to triglyceride resynthesis during times of energy mobilization in the fat cell.

**MATERIALS AND METHODS**

**Animal treatment and care.** Wild-type and aP2-PTG transgenic mice were maintained on the CD-1 background. For the current studies, only male mice were utilized. Mice were maintained on a 12:12-h light-dark cycle and allowed free access to standard chow and water under specific, pathogen-free conditions in the Carlson Barrier Facility at the University of Chicago. All mice procedures were approved by the University of Chicago Institutional Animal Care and Use Committee. Mice were weaned at 3 wk of age, and tail clippings were acquired for genotyping via PCR (16). For studies comparing fed vs. fasted conditions, animals had free access to either standard chow and water or chow, and bedding was removed for an overnight period of 16 h (5 PM to 8 AM). For all studies, animals 2.5 mo of age were used and euthanized via CO2 narcosis. Blood samples were obtained immediately via cardiac puncture. Epididymal adipose tissue and liver were harvested and either used immediately or frozen on dry ice and stored at −80°C for future study.

For the weight gain/loss protocol, wild-type and transgenic 1.5-mo-old male mice were fed either standard chow diet or a high-fat diet (45% calories from fat; Research Diets, New Brunswick, NJ) for 5 wk. Upon completion of the high-fat feeding, all mice were fed a standard chow diet for an additional 5 wk. Animals were weighed three times weekly over the entire 10-wk time course.

For the metabolic cage analysis, 2.5-mo-old mice were used. Each mouse was weighed and placed into an individual closed metabolic cage (TSE Instruments, Chesterfield, MO) for 7 days with free access to H2O and food consumption. Data collected were typical of metabolic phenotyping, including H2O and food consumption, VO2, VCO2, respiratory exchange ratio (RER), energy expenditure, and movement around cage. Data points were collected every 15 min via the Lab Master System software (TSE Instruments) and imported into Microsoft Excel XP for analysis. Each mouse was fasted for approximately the final 15 h of analysis. Mean data points per genotype for each hour fasted were analyzed and are reported.

**RNA and protein analysis.** To perform real-time quantitative RT-PCR, RNA was isolated from 300 mg of epididymal adipose tissue from wild-type and transgenic mice via the TriZol method (Invitrogen). To produce cDNA, RT-PCR was performed with an iScript cDNA synthesis kit (Bio-Rad). Real-time quantitative PCR was performed as described previously (16) with iCycler and MyiQ software for data analysis (Bio-Rad). The following primers were utilized: control 18S (sense GCTGGAATTACCGCCGCT, antisense CGGCTACACATCCAAGGA) and PEPC-K-C (sense AGAACAAGGAGTGGAGACCGC, antisense TCAATAATGGGGCACTCGGCT). Cycling parameters were 3 min at 95°C, 40 cycles of 10 s at 95°C, 30 s at 60°C, and 30 s at 72°C.

For protein analysis, immunoblotting was performed as described (16). Briefly, tissues of 2.5-mo-old male mice were harvested and quickly minced with scissors. Tissues were next lysed with a glass Dounce homogenizer in homogenization buffer [50 mM HEPES (pH 7.4), 150 mM NaCl, 1 mM EDTA, 10 mM NaF, 10% glyceral, and protease inhibitors]. Samples were then transferred to eppendorfs and nutated for 2 h at 4°C and then centrifuged at maximum speed for 20 min. Protein concentrations for equal sample loading were determined by Bradford assay (Bio-Rad). Samples were resolved by SDS-PAGE and transferred to PVDF membranes (Millipore, Billerica, MA), and immunoblotting (17) was performed with antibodies against anti-PEPCK-C (P-14; Santa Cruz Biotechnology, Santa Cruz, CA) and anti-β-actin (A5441; Sigma, St. Louis, MO).

In vitro NEFA, glycerol, and lactate measurements. Wild-type and transgenic epididymal fat was harvested from animals fed ad libitum. The fat was immediately minced into 100-mg pieces and placed into glass scintillation vials containing 37°C Krebs-Ringer bicarbonate-HEPES (KRHB) supplemented with 5 mM glucose and 3% BSA-free fatty acid free (Sigma) at pH 7.4 with or without 10 μM DL-Isoprterenol hydrochloride (Sigma). Samples were incubated at 37°C for 60 min with gentle agitation. Following the time course, incubation medium was removed, and released NEFA levels (Wako Diagnostics, Richmond, VA) and glycerol (Sigma) released from adipose tissue were determined according to the manufacturers’ instructions. Lactate measurements were made based upon the method of Hohorst (14) with modifications. A 50-μl aliquot of KRHB from the assay described above was collected and added to a reaction cocktail [0.33 mg/ml glucose-0.27 M hydroxylamine, NAD+, and LDH (Sigma)] and incubated at 37°C in a shaking H2O bath for 45 min. Lactate levels between untreated and treated conditions were determined relative to lactate standards (Sigma). In vitro ATP measurements. Epididymal adipose tissue was harvested from wild-type and transgenic mice fed ad libitum. Five hundred milligrams of tissue was placed into glass scintillation vials containing KRHB supplemented with 5 mM glucose and 3% BSA-free fatty acid free (Sigma). Samples were incubated at 37°C for 120 min with gentle agitation. Following the time course, samples were placed immediately on ice before being placed into 1 ml of ATP assay buffer (100 mM Tris, 4 mM EDTA, pH 7.75) and homogenized on ice. Homogenized samples were then boiled for 5 min, placed on ice for 5 min, and centrifuged 1,000 g for 60 s. Infranant was collected and used for ATP determination via an ATP Bioluminescence Assay Kit CLS II (Roche, Madison, WI) and protein determination via Bradford assay.

**Blood analysis.** Blood samples were obtained immediately via cardiac puncture following fed vs. fasting overnight period. Eighty microliters of EDTA (10 mg/ml) was added to each sample and then centrifuged at 2,300 g at 4°C for 5 min to separate serum. Serum levels of NEFAs and glycerol were determined as described above. To determine serum lactate values, 200 μl of serum was added to 100 μl of aceton, vortexed, and centrifuged at 8,000 g for 10 min. Fifty microliters of supernatant was then incubated with 1 ml of reaction cocktail [0.33 M glycine-0.27 M hydroxylamine, NAD+, and LDH (Sigma)] at 37°C in a shaking H2O bath for 45 min, following which lactate levels were determined as described previously.

**Statistical analysis.** The data shown represent means ± SE. Data were compared using unpaired, two-tailed Student’s t-tests and analyses performed with Microsoft Excel XP. A P value ≤0.05 was considered statistically significant.

**RESULTS**

Adipose tissue lactate production is increased coordinately with glycogenolysis. Previous characterization of the aP2-PTG transgenic mouse model demonstrated that the sustained elevation in adipose tissue glycogen is dynamically regulated, with marked mobilization occurring during an overnight fast (16). The metabolic fate of the released glucose 1-phosphate and the potential interplay between glycogenolysis and lipolysis were investigated. Initially, the increased flux of substrate through glycolysis following lipolytic stimulation that could modulate lactate production from the adipose tissue was examined. Epididymal adipose tissue was harvested from wild-type and transgenic mice and placed into KRHB buffer and treated with ±10 μM isoprotrenol to stimulate glycogenolysis.
Following a 60-min incubation, lactate release into the assay buffer was determined in parallel with adipose glycogen (data not shown). In vitro levels of lactate produced were significantly elevated in the transgenic tissue in both the basal and isoproterenol-treated conditions (Fig. 1A). Each condition demonstrated an ∼300% increase in lactate production from transgenic adipose tissue relative to wild-type tissue. These results indicate that glycogen turnover is dynamic, occurring in the basal state and to a significantly greater extent following isoproterenol stimulation. Although adipose tissue lactate generation was elevated in the transgenic model, there were no significant changes in levels of serum lactate between genotypes under fed or fasted conditions (Fig. 1B). It is interesting to speculate whether or not the adipose tissue-produced lactate is utilized metabolically by the liver (36). However, such elevated in vitro levels do support the notion that adipose tissue glycogenolysis in a fasted state results in an elevated release of glycogen-derived glucose into glycolysis, producing substrate that may be utilized by liver (12, 36).

Alterations of ATP content in aP2-PTG adipocytes. In addition to lactate levels, ATP content between wild-type and transgenic adipose tissue was examined in vitro. It was hypothesized that the elevated glycogenolysis could result in altered ATP production. Adipose tissue was harvested from fed wild-type and transgenic animals and placed into KRBH buffer ± 10 μM isoproterenol. As seen in Fig. 2, basal levels of ATP were ∼9% lower in transgenic adipose tissue compared with the wild-type tissue, although this difference did not reach statistical significance. Previous work had shown that isoproterenol stimulation decreases cellular AMP/ATP levels (10). Surprisingly, the decrease in ATP content observed following treatment with isoproterenol was significantly greater in the transgenic tissue. These results are extremely intriguing. The data suggest that the transgenic adipose tissue may be either producing less ATP or consuming more ATP than wild-type tissue. The elevation of lactate production relative to a decrease in ATP content under lipolytic conditions suggests that the transgenic adipose tissue has adapted to the elevated glycogen shifting toward glycolytic rather than oxidative metabolism (35, 39).

NEFA levels are significantly decreased in aP2-PTG mice. It is well known that a percentage of free fatty acids liberated during lipolysis are continuously reesterified back into triglyceride to prevent systemic lipotoxicity (15, 38). Known as the fatty acid (FA)-triglyceride cycle, the induced expression of PEPCK-C in adipocytes during prolonged fasting allows for glyceroneogenesis and the generation of G3P for reesterification of fatty acids during lipolysis, providing a break on NEFA release (26). Utilizing the aP2-PTG transgenic mice, the potential interplay between glyceroneogenesis and the FA-triglyceride cycle was examined. Epididymal adipose tissue was harvested from each genotype and placed into KRBH buffer ± 10 μM isoproterenol. Following a 60-min incubation at 37°C, levels of released NEFAs were determined. As shown in Fig. 3A, in vitro release of NEFAs from transgenic adipose tissue was significantly decreased relative to wild-type tissue under both basal and isoproterenol-treated conditions, whereas there was no change in glycerol release (Fig. 3B). There were also no detectable changes in the expression of hormone-sensitive lipase or adipose triglyceride lipase measured by quantitative analysis.
real-time PCR (Supplemental Fig. S1; Supplemental Material for this article can be found on the AJP-Endocrinology and Metabolism web site). Thus, the differential changes in NEFA and glycerol release are likely explained through an increased reesterification of fatty acids into triglyceride in the transgenic mouse adipose tissue (9). These findings were recapitulated at the in vivo level following NEFA and glycerol measurements from serum in the fed vs. fasted states (Fig. 3, C and D). Therefore, it is possible that elevated mobilization of glycogen into the glycolytic pathway is being diverted into the production of G3P in addition to being metabolized to lactate. This G3P would then provide the backbone needed for the observed increased reesterification of fatty acids in the transgenic animals.

**Induction of PEPCK-C expression upon fasting is blunted in adipose tissue of aP2-PTG mice.** Previous work has shown that glycogenolysis is increased in adipose tissue during a prolonged fast through the induction of PEPCK-C (1, 27, 29, 30). Accordingly, levels of PEPCK-C expression in wild-type and transgenic adipose tissue were examined by quantitative real-time PCR analysis in the fed state and following a 16-h fast. Analysis of PEPCK-C mRNA levels were significantly blunted in the aP2-PTG mice (Fig. 4A). Furthermore, there was also decreased expression of PEPCK-C protein in the adipose tissue of fasted transgenic animals compared with wild type (Fig. 4B). As a control measurement, quantitative real-time PCR was also performed with liver tissue harvested from both genotypes following fed and fasted periods. The expected upregulation of liver PEPCK-C under fasted conditions was observed in each genotype, indicating that altered PEPCK-C expression was specific to transgenic adipose tissue (Fig. 4C) and likely arose from increased energy flux provided by glycogenolysis. Previous work has demonstrated that PEPCK expression in adipose tissue is transcriptionally regulated by extracellular stimuli during prolonged fasting (13). The current results suggest that intracellular metabolic cues must also be detected by the adipocyte so that when sufficient G3P arises from glycogenolysis to support triglyceride resynthesis, the redundant expression of PEPCK-C is reduced.

**Whole body substrate utilization is unchanged in aP2-PTG mice.** Next, indirect calorimetry was utilized to investigate whether increased glycogenolysis at the level of adipose tissue altered whole body substrate utilization. It has been demonstrated in the literature that adipose tissue harvested from a rodent upon feeding following a prolonged fast demonstrated
an elevated respiratory quotient, indicating utilization of physiologically elevated glycogen (22). It was hypothesized in this study that an increased fasting RER closer to 1.0 would be detected in transgenic animals, indicating that they were relying more heavily on glucose oxidation stemming from adipose tissue rather than lipid oxidation, as occurs normally when they are fasted. Wild-type and transgenic mice were weighed and placed into individual metabolic cages for 7 days total. On days 1–6, animals had free access to standard chow. Each animal was then fasted overnight for approximately the final 15 h of the study. Although the transgenic RER trended slightly higher than wild-type fasting RER values, overall no statistically significant differences were found (Fig. 5A). Additionally, no significant differences were detected between genotypes regarding RER in the fed ad libitum state (Fig. 5B). Analysis of energy expenditure (kcal·h⁻¹·kg⁻¹) was also performed, and no differences between genotypes under fed and fasted conditions were detected (data not shown). Thus, under these conditions, it does not appear that elevation of glycogen storage and subsequent mobilization in adipose tissue markedly affect whole body substrate utilization.

aP2-PTG mice are resistant to weight loss. Previous work demonstrated no differences in weight gain between wild-type and aP2-PTG mice fed either a standard chow or high-fat diet (16). Given the alterations in NEFA release and triglyceride resynthesis in the aP2-PTG mice, the potential impact of elevated glycogen storage on weight loss following cessation of high-fat feeding was investigated. Male aP2-PTG and wild-type control littermates underwent high-fat feeding (45% of calories from fat) for 5 wk. Both genotypes gained ~44% body weight during the protocol, and weight gain between the two genotypes was not statistically different. Under this protocol, there was no significant increase in fasting plasma insulin levels for either genotype, indicating that the high-fat feeding protocol was not sufficiently long to induce systemic insulin resistance (data not shown). Next, all mice were returned to a standard chow diet for an additional 5-wk period, which has previously been shown to result in significant weight loss (6, 28). Over this time frame of chow feeding, the wild-type animals displayed a steady loss of weight (Fig. 6A). In contrast, weight loss in the transgenic animals on the chow diet was significantly blunted over the entire 5-wk span (Fig. 6B). Although there was individual variability, there was a highly significant difference in average weekly weight loss between the two genotypes, with wild-type animals losing an average of 4% of their weight each week, whereas transgenic animals lost ~1%/wk (Fig. 6B). There were no significant differences in serum glucose and insulin levels between the genotypes, although insulin trended higher in the transgenic animals (Supplemental Fig. S2). Additionally, there was no detectable difference in insulin signaling in primary adipocytes in vitro (Supplemental Fig. S3), indicating that the weight difference between the genotypes was not large enough to alter global insulin sensitivity or energy metabolism. However, since there were no detectable differences in energy expenditure or food consumption between the two genotypes during indirect calorimetry analysis (Fig. 5), the weight loss results suggest that
enhanced glycogen levels prevent triglyceride mobilization even under conditions of caloric reduction.

DISCUSSION

Adipose tissue fulfills a crucial physiological role through the storage and mobilization of energy. Postprandially, insulin triggers glucose uptake into the adipocyte via the GLUT4 transporter for metabolism into glycolysis, the pentose phosphate pathway, oxidative metabolism, or incorporation into glycogen. Dietary lipid is also taken into the adipocyte and assembled into triglyceride for long-term storage. Dihydroxyacetone phosphate produced during glycolysis may be further reduced to G3P, providing a glycerol backbone for the esterification of three fatty acids into triglyceride, thus inherently linking carbohydrate and lipid metabolism. In a catabolic state, catecholamines elicit energy mobilization, stimulating the hydrolysis of triglyceride. Adipose glycogen levels, already scant in the fed state, are further reduced. The underlying mechanisms responsible for the dynamic regulation of adipose glycogen metabolism and its true physiological role in times of energy storage vs. mobilization remain unclear.

In the first half of the 20th century, adipose tissue had been considered a passive receptacle for lipid produced by the liver while being devoid of any metabolic machinery. In 1942, researchers noticed that there was a massive, transient spike in glycogen levels in adipose tissue following refeeding of rats that had undergone a chronic partial fast (37). Since it was known that glycogen could not pass through the plasma membrane, the authors concluded that adipose tissue “constitutes in fact a regulated organ with a definite role to play in the metabolism of carbohydrates and lipids” (37). Thus the discovery that adipocytes are metabolically active came from glycogen rather than lipid research. Recently, these classical studies have been recapitulated through the use of chronic leptin administration to promote weight loss and depletion of adipose mass followed by immediate leptin withdrawal and refeeding (3). In conjunction with this state of acute hypoleptinemia, a marked temporary glycogen accumulation that preceded lipid synthesis and the reaccumulation of triglyceride stores in the adipose mass was observed. The role that adipose glycogen synthesis plays in the regulation of lipid metabolism remains incompletely understood.

We have previously generated a transgenic mouse model in which the expression of the protein phosphatase-1 glycogen targeting subunit PTG is under the control of the aP2 promoter (16). There was a relatively modest increase in glycogen-targeted phosphatase levels but a marked increase in the dephosphorylation of glycogen synthase and phosphorylase.

Fig. 6. Impaired weight loss in the aP2-PTG transgenic animals. 1.5-mo-old mice of each genotype were placed on a high-fat diet for 5 wk. Mice were then returned to a standard chow diet for the next 5 wk. Mice were weighed 3 times weekly throughout the study. %Change in body weight from the previous week (A) or average weight loss per week (B) was calculated. The values shown represent means ± SE per genotype for 9 mice. ***P ≤ 0.001; *P ≤ 0.05.
resulting in glycogen levels that were 100- to 400-fold elevated vs. control mice (16). The glycogen stores were stably elevated for more than 1 year and could be mobilized during an overnight fast, indicating that a new “set point” for glycogen metabolism had been established, as opposed to a glycogen storage disease. Despite the hyperaccumulation of carbohydrate storage, there were no detectable alterations in lipid synthesis in vitro, nor was there any difference in weight gain on chow or high-fat diets. These results demonstrated that adipose tissue was physiologically capable of storing levels of glycogen far higher than occur normally, and by inference, adipose glycogen is maintained at low levels through active regulation of gene expression rather than through a passive default mechanism. Thus, two questions remain unanswered: why are glycogen stores so far below levels found in skeletal muscle and liver, and why do they spike during the transition from chronic fasting to refeeding?

In the current study, the fate of the mobilized glycogen in adipocytes from the aP2-PTG mice was studied. Adipocytes isolated from rats starved for 48 h have demonstrated a marked shift in glucose metabolism, yielding greater levels of lactate (36). In this study, lactate generation from transgenic adipose tissue was elevated over wild type in both the basal and isoproterenol-stimulated states. The elevated production of lactate in the basal state suggests that enhanced turnover of glycogen stores occurs in the transgenic adipocytes despite a lack of adrenergic stimulation. Despite the dramatic increase of adipose tissue-derived lactate, no changes in serum lactate levels were observed between the genotypes, suggesting that it is a minor constituent of circulating lactate. However, previous work with human subcutaneous adipose tissue revealed that lactate production increased following an oral glucose load, whereas venous blood levels remained unchanged (12). The metabolic fate of this released lactate, particularly if it can be utilized by the liver, remains to be investigated.

Another metabolic fate of the glucose 1-phosphate produced during glycogenolysis would be the production of G3P for use in NEFA reesterification (Fig. 7). Interestingly, NEFA release from transgenic adipose tissue was reduced in the basal and isoproterenol-treated conditions. In contrast, there was no corresponding change in glycerol release between the two genotypes. The alteration in the NEFA/glycerol ratio strongly suggests that there is increased triglyceride resynthesis due to the simultaneous increase in glycogen breakdown induced by lipolytic stimuli. As was the case with lactate production, there were significant differences in the basal state, suggesting that the elevation of tonic glycogen turnover is impacting these two metabolic pathways, and the effects are further exaggerated upon isoproterenol stimulation. Normally, during fasting in mice, PEPCK-C expression is markedly increased not just in liver to promote gluconeogenesis but also in adipose tissue to catalyze glyceroneogenesis (13). Adipocytes, PEPCK-C activity contributes to the reesterification of 30–40% of the NEFAs released during lipolysis (1, 15, 27, 30, 38), which plays a critical role in applying a brake to chronic lipolysis and the potential induction of global lipotoxicity. Historically, glycogen has been ignored as a suitable carbon source for reesterification of NEFA during lipolysis, although several studies have linked glycogen to the synthesis of triglyceride following cessation of a fasted state (32–34). In aP2-PTG mice, the elevated glycogen stores provide G3P to promote NEFA reesterification, which is similar to previous reflecting obtained using a transgenic model overexpressing PEPCK in adipose tissue (9). Interestingly, in the current study the data suggest that the transgenic adipocytes were also able to “sense” the metabolic flux from glycogen to G3P and prevent extracellular stimuli from inducing the redundant transcriptional expression of PEPCK-C in fasting animals. The molecular mechanisms by which PEPCK-C levels were blunted in the transgenic adipose tissue remain to be determined.

A respiratory quotient greater than 1.0 was demonstrated previously in adipose tissue from animals subjected to the fasting/feeding protocol in which the physiological spike in glycogen had occurred (22), indicating pure carbohydrate metabolism within the fat. Accordingly, indirect calorimetry was performed on control and aP2-PTG transgenic mice to examine altered glycogen storage-impacted energy utilization by other tissues. No statistically significant differences in RER were observed during the 24-h cycle of ad libitum feeding. Although initial characterization of the aP2-PTG model reported no significant difference in circulating free fatty acids (16), there were differences that approached significance, and analysis in the current study focused on the measurement of NEFA. In parallel to the in vitro measurements, there was also a dramatic decrease in circulating NEFA following an overnight fast of aP2-PTG vs. control animals, with no corresponding alteration in plasma glycerol levels. To investigate the physiological significance of these results, control and aP2-PTG animals were subjected to an overnight fast in the metabolic cages. Both genotypes displayed the expected decline in RER over time, reflecting a fasting-induced shift toward global lipid utilization (2). Although the fasting RER of transgenic animals trended slightly above the wild-type group, no statistically significant differences were detected. Cumulatively, these results indicate that the reduction of NEFA release from the transgenic adipose tissue was not large enough to negatively
impact fatty acid oxidation in other tissues during a prolonged fast.

However, the issues of why adipose tissue has low glycogen stores when it is physiologically capable of accommodating far higher levels and what purpose glycogen spiking serves in adipocytes following cessation of a fast have remained. The results from the weight gain/loss protocol may provide some novel insights. Following 5 wk of high-fat feeding, there was no significant difference in weight gain in control and aP2-PTG mice, as had been reported previously (16). However, following replacement of the high-fat diet with standard chow, there was a dramatic difference in weight loss between control and aP2-PTG mice that was not evident on standard chow or high-fat feeding (16). This intriguing result suggests that, upon expansion of adipose tissue mass during caloric excess, the elevated glycogen levels in the transgenic animals inhibit the mobilization of triglyceride and weight loss following the return to chow feeding. This result might also shed some light on why, following chronic partial fasting and weight loss, refeeding induces a transient marked elevation of glycogen that may serve to both put a rapid brake on lipolysis and also allow the adipocyte to metabolically reset and shift toward triglyceride synthesis after an extended state of lipolysis. However, it should be noted that glycogen levels in the adipose tissue of aP2-PTG mice are far in excess of levels found in wild-type animals, so the precise physiological significance of these findings is not yet clear. Clearly, further work is necessary to fully elucidate the physiological role of glycogen in adipose tissue and its potential cross-talk with lipid metabolism.

GRANTS

This work was supported by National Institutes of Health (NIH) Grant R01-DK-064772 (to M. J. Brady) and a predoctoral fellowship from the NIH Research Training Grant in Cardiovascular Pathophysiology and Biochemistry Training Program (T32-HL-007237; to K. R. Markan). Work done with the metabolic cage analysis was supported by the Diabetes Research and Training Center (P60-DK-20595) and a gift from the Kovler Family Foundation (to H. Ye).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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