Glucose induces expression of stearoyl-CoA desaturase in 3T3-L1 adipocytes

Brynn H. JONES*†, Melissa K. STANDRIDGE*, Kate J. CLAYCOMBE*, Pamela J. SMITH‡ and Naïma MOUSTAÏD-MOUSSA*†¹

*Department of Nutrition, University of Tennessee, Knoxville, TN 37996-1900, U.S.A., †Physiology Program and Agricultural Experiment Station, University of Tennessee, Knoxville, TN 37996-1900, U.S.A., and ‡Ross Laboratories, Columbus, OH 43215-1724, U.S.A.

Stearoyl-CoA desaturase (SCD; EC 1.14.99.5) is a key enzyme in the synthesis of polyunsaturated fatty acids. Liver and adipose tissue are the predominant sites of SCD expression. Regulation of hepatic SCD by various nutritional and hormonal factors, such as insulin, dietary carbohydrates and polyunsaturated fatty acids, has been well documented. Little is known, however, about adipocyte SCD regulation despite high levels of SCD activity in adipose tissue. The present study was designed to

investigate SCD regulation in adipocytes by examining the effects of glucose and insulin on SCD expression. We report here that glucose availability directly increased SCD gene transcription in 3T3-L1 adipocytes. This response was independent of insulin, and insulin alone in the absence of glucose had no effect on SCD mRNA levels. SCD thus represents a novel model in which to investigate the mechanisms of direct regulation of gene expression by glucose in adipose cells.

INTRODUCTION

Stearoyl-CoA desaturase (SCD; EC 1.14.99.5) catalyses the initial desaturation of long-chain fatty acids, primarily palmitate and stearate, at the Δ^9 position. The reaction catalysed by SCD, which occurs predominantly in liver and adipose tissue, represents the first regulatory step in the formation of long-chain unsaturated fatty acids [1]. Changes in the activity of SCD in tissues are reflected in cell membrane phospholipid composition [2]. SCD, therefore, has the potential to regulate a variety of key physiological variables, such as insulin sensitivity [3] and metabolic rate [4], which have been shown to be influenced by the membrane fatty acid profile.

Regulation of SCD enzyme levels is exerted primarily through changes in the transcriptional rate of two SCD genes (*SCD1* and *SCD2*), each of which encodes the same functional enzyme [5]. Studies of hepatic SCD expression have indicated that insulin and dietary carbohydrates interact to control expression of the SCD gene *in vivo*. Fasting and refeeding decreased and restored, respectively, SCD mRNA levels in normal, but not in diabetic, mice [5]. SCD mRNA levels were dramatically lower in the livers of diabetic mice; insulin and dietary fructose synergized to maximally restore SCD mRNA levels more effectively than either treatment alone [5]. Polyunsaturated fatty acids (PUFAs) have also been shown to decrease hepatic SCD expression *in vivo* and *in vitro* [6–8].

Less is known about the regulation of SCD in adipose tissue, despite high levels of SCD enzyme activity in this tissue. We have reported [6] that SCD was overexpressed in adipose tissue of obese Zucker rats. We further demonstrated that polyunsaturated fatty acids decreased SCD expression in rat adipose tissue *in vivo* and in 3T3-L1 adipocytes *in vitro* [6], similar to the effects of PUFAs on SCD expression in liver. It is unknown, however, whether carbohydrate regulation of SCD as reported in liver can also be extended to adipose tissue. Several other genes involved

in lipid metabolism, such as those encoding fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC), have been shown to be regulated by carbohydrate in adipose tissue [9]. Glucose was shown to increase FAS expression independent of insulin-mediated effects, indicating that carbohydrates may directly control the transcription rate of lipid-metabolizing enzyme genes in adipose tissue [9]. Whether other genes involved in lipid metabolism, such as those encoding SCD, are subject to similar regulation by glucose in adipocytes has not previously been addressed. We therefore designed the present study to examine adipose tissue regulation of SCD by glucose availability using the 3T3-L1 adipocyte cell line as a model. We report here that SCD is upregulated by glucose in an insulin-independent manner.

MATERIAL AND METHODS

Cell culture

3T3-L1 preadipocytes were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum and antibiotics (standard medium). At confluency, cells were induced to differentiate as described previously according to a modification [10] of the method of Rubin et al. [11]. Briefly, the medium was supplemented at confluency (day 0) with 250 nM dexamethasone and 0.5 mM isobutylmethylxanthine for 2 days, after which the cells were returned to standard medium. Differentiation was considered to be complete at day 5, at which time the cells were incubated in glucose-free (with 1 mM sodium pyruvate added as an energy source), serum-free medium containing 1% (w/v) bovine serum albumin for 16 h. Cells were then switched to glucose-free, serum-free media supplemented with glucose (5 mM or 25 mM) or fructose (25 mM) or 2-deoxyglucose (2-DG; 1 mM or 25 mM) or maintained in glucose-free, serum-free medium for 24 h. Experiments with glucose were conducted both in the absence and presence of insulin (50 nM). Time-course

Abbreviations used: ACC, acetyl-CoA carboxylase; ANOVA, analysis of variance; 2-DG, 2-deoxyglucose; DMEM, Dulbecco's modified Eagle's medium; FAS, fatty acid synthase; PUFA, polyunsaturated fatty acid; SCD, stearoyl-CoA desaturase.

¹ To whom correspondence should be addressed (e-mail moustaid@utk.edu).

analysis was conducted by treating cells with or without glucose (25 mM) for 0–48 h.

RNA analysis

At the termination of incubations (described above) cells were harvested for RNA isolation using the guanidine/CsCl method, as described previously [10]. Individual culture dishes (100 mm) of cells were used for each RNA sample within treatments, and three to four samples were included in each treatment group in an experiment. All experiments were repeated three to five times.

RNA from all samples within an experiment was electrophoresed on the same agarose gel, transferred to positively charged nylon membranes and analysed by Northern blotting. Membranes were hybridized with 32 P-labelled cDNA probes for murine SCD1 [12] and β -actin (A.T.C.C.) that were labelled by the random primer method. After washing under stringent conditions, membranes were exposed to X-ray film (New England Nuclear, Boston, MA, U.S.A.). Autoradiograms were analysed by densitometric scanning. Results are reported in arbitrary densitometric units as ratios of SCD mRNA to β -actin mRNA.

Transcription rate assays

Transcription rate assays were performed in nuclei isolated from 3T3-L1 adipocytes in control (glucose-free) or glucose (5 mM and 25 mM) treatment conditions. Nuclei were isolated from 3T3-L1 adipocytes as described previously [10]. Briefly, cells were homogenized in lysis buffer containing detergent (Nonidet P40) and nuclei were isolated by centrifugation. Nuclear run-on assays and hybridizations were then conducted as described previously for 3T3-L1 adipocytes [10]. Labelled RNA was hybridized with plasmids containing cDNAs encoding SCD1 [12] and β -actin. Membranes were exposed to film and autoradiogram results were quantified by scanning laser densitometry.

Statistical analysis

Analysis of variance (ANOVA) was used to compare overall group means. A significant *F*-test was followed by the Bonferroni multiple comparisons procedure to compare differences between pairs of group means [13]. All tests were conducted using a 95% confidence interval. Values are shown as means $\pm$ S.D.

RESULTS AND DISCUSSION

This series of experiments was initiated to investigate the effects of carbohydrates on the expression of the SCD gene in adipocytes. The investigation of this issue began by determining whether glucose availability increased SCD expression in differentiated 3T3-L1 adipocytes. The effect of 48 h of glucose treatment on SCD expression (measured as the ratio of SCD mRNA to β -actin mRNA) in glucose-deprived 3T3-L1 adipocytes was determined. ANOVA revealed a significant effect of media glucose concentration on SCD mRNA levels in 3T3-L1 adipocytes ($F = 8.001$; $P < 0.01$). *Post hoc* comparisons (Bonferroni) indicated that both 5 mM and 25 mM glucose concentrations were effective in significantly upregulating SCD expression ($P < 0.05$); ratio values were 0.401 ± 0.035 and 0.479 ± 0.116 , respectively, and 0.180 ± 0.018 for the control, $n = 4$. The magnitude of SCD upregulation was approximately 20% greater with 25 mM glucose (266% of control levels) than with 5 mM glucose (222% of control values), although this difference was not statistically significant. This pattern of SCD responsiveness to glucose is

Table 1 Absence of an effect of insulin on SCD expression

Cells were treated for 48 h; $n = 3$. Data represent the ratio of SCD mRNA to β -actin mRNA.

	– Glucose	+ Glucose (25 mM)
– Insulin	0.335 ± 0.091	0.824 ± 0.077
+ Insulin (50 nM)	0.270 ± 0.039	0.876 ± 0.175

similar to regulation of two other lipid-metabolizing enzyme genes, FAS and ACC, both of which were induced by glucose in adipose tissue explants [9]. To assess the time frame necessary for SCD responsiveness to glucose, we conducted a similar experiment in which 3T3-L1 adipocytes were treated with 25 mM glucose for periods ranging from 0 to 48 h. The effect of glucose on SCD mRNA levels was quite rapid: 6 h of glucose treatment was sufficient to elicit 87% of the maximal response seen after 48 h of glucose treatment (0.346 ± 0.073 , time zero; 0.760 ± 0.052 , 6 h; 0.824 ± 0.078 , 48 h). These data are the first to indicate that glucose availability directly regulates SCD expression in any cell type. Refeeding a high-carbohydrate, fat-free diet to fasted mice has been shown to maximally restore hepatic SCD expression compared with refeeding a chow diet [8]. However, in these studies it is difficult to separate the effects of high levels of carbohydrates from the absence of suppressive actions of PUFAs which have been shown to inhibit SCD expression [6,7].

The effects of glucose on expression of other lipid-metabolizing enzyme genes in adipose tissue has been shown to be independent of, but enhanced by, insulin [9]. *In vivo*, insulin was also able to partially restore SCD mRNA levels in the livers of diabetic mice [5]. Insulin has also been shown to increase SCD activity in isolated chicken hepatocytes [14] and to increase the transcription rate of the *SCD1* gene in the H2.35 mouse liver cell line [15]. The experiments described above were conducted in the complete absence of insulin (in a serum-free medium). Glucose regulation of gene expression has been reported to be either insulin-dependent or insulin-independent. Therefore in order to determine whether insulin enhanced the effects of glucose alone or whether insulin itself increased SCD expression in adipose cells, 3T3-L1 adipocytes were treated with or without glucose (25 mM) in the presence or absence of insulin (50 nM) for 48 h. The results of these experiments are reported in Table 1. Two-way ANOVA indicated that insulin had no effect on SCD mRNA levels in 3T3-L1 adipocytes in either the absence or presence of glucose. This analysis also indicated that glucose and insulin did not interact to control SCD expression. These data demonstrate that SCD expression is not directly regulated by insulin in adipose cells. Although numerous other genes involved in lipid metabolism have been shown to be regulated by insulin in adipocytes [16], no previous reports have addressed the effects of insulin on SCD expression in adipose cells. We reported previously that insulin increased the transcription rate of SCD in adipocytes; however, pharmacological insulin concentrations (2 μ M) were required, indicating an effect mediated through the insulin-like growth factor-1 receptor rather than through the insulin receptor [12]. Interestingly, although insulin synergizes with glucose to increase expression of FAS and ACC in adipose tissue [9], insulin did not significantly enhance the effects of glucose on SCD expression. The actions of glucose on SCD expression in 3T3-L1 adipocytes thus appear to be independent of insulin-regulated processes such as glucose transport via the GLUT4 glucose transporter or insulin-sensitive steps in glycolysis.

Figure 1 Effects of various carbohydrates on FAS and SCD mRNAs

Abbreviations: NG, no glucose (pyruvate-supplemented medium); G, glucose; 2-DG, 2-deoxyglucose; F, fructose. Concentration of each carbohydrate = 25 mM, control = 0 mM glucose; all treatments were carried out in serum-free media for 24 h. Densitometric scanning of the autoradiogram signals from five different experiments gave the following ratios of SCD mRNA/ β actin mRNA, when the control values were set to 100%: glucose 290 ± 35 , 2-DG 13 ± 1 , fructose 81 ± 9 , where glucose and 2-DG values are significantly different from control values ($P < 0.05$).

Transcription rate assays were conducted to identify the molecular mechanisms responsible for glucose-induced SCD expression in 3T3-L1 adipocytes. Data from densitometric scanning of autoradiogram signals generated from the run-on assays and expressed in arbitrary units are 0.21 for controls, 0.81 for 5 mM glucose and 0.97 for 25 mM glucose. This indicated that both 5 mM and 25 mM glucose significantly increased SCD transcription by approximately 4–5-fold over glucose-free control levels. These actions were specific to SCD, as the transcription rate of another gene (angiotensinogen) expressed in adipocytes was not affected by glucose concentration (results not shown). Nutritional control of SCD expression in liver has been attributed to both transcriptional and post-transcriptional mechanisms [5,7]. Although we did not measure mRNA stability in this study, the magnitude of glucose effects on transcription accounted for its actions on SCD mRNA levels. Based on these data, we conclude that upregulation of SCD expression in 3T3-L1 adipocytes is due to the increased transcription rate of the SCD gene.

We then wanted to determine what component(s) of glucose metabolism were necessary for increased SCD expression in glucose-treated adipocytes. For these experiments 3T3-L1 adipocytes were treated with other carbohydrates that represent early steps in the glycolytic pathway. 2-DG is an isomer of glucose that is transported into the cell and phosphorylated but not metabolized further. Fructose enters carbohydrate metabolism as fructose 6-phosphate after hexokinase phosphorylation and is metabolized through the remaining steps of glycolysis. Limited studies have investigated fructose metabolism in adipocytes; however, a few groups have reported fructose regulation of glucose transport [17], glucose metabolism [18] and glucose-regulated proteins [19] in adipose cells. Unlike glucose, none of the other carbohydrates tested increased SCD expression in 3T3-L1 adipocytes (Figure 1); FAS, a glucose-regulated lipogenic gene, was used as a control. Figure 1 shows that both mRNAs are increased by glucose; however, SCD was more sensitive than FAS to glucose regulation in 3T3-L1 cells. Surprisingly, 2-DG did not stimulate FAS mRNA content, whereas it significantly reduced SCD mRNA content. These differences were not due to RNA loading, as indicated by the 18 S RNA band. SCD mRNA

levels in fructose-treated 3T3-L1 adipocytes were not significantly affected by glucose compared with control cells (glucose-free). These results are in contrast to the effects of fructose on hepatic SCD expression *in vivo*, in which fructose alone was able to partially restore SCD expression in streptozotocin diabetic mice [5]. However, this difference may be due to differences in fructose metabolism between adipocytes and hepatocytes.

Previous studies describing glucose regulation of FAS and ACC in adipose tissue demonstrated that hexose phosphate was the critical regulatory intermediate in glucose actions on lipogenic gene expression [9]. In the present study, however, we did not observe any upregulation of SCD expression by 2-DG. In fact, SCD expression was significantly lower in 2-DG treated adipocytes than in control (glucose-free) cells. We repeated these experiments using lower concentrations of 2-DG (1 mM) and again found reductions in SCD expression compared with controls. We also failed to detect an induction of the FAS mRNA by 2-DG in 3T3-L1 adipocytes, whereas this mRNA was stimulated by this analogue in isolated primary adipocytes (results not shown), as reported previously [9]. Our findings on SCD and FAS regulation in 3T3-L1 adipocytes thus differ from those reported for FAS and ACC in isolated adipocytes [9] and the reason for this discrepancy remains to be investigated. It also remains to determine the effector molecule(s) in glucose regulation of these genes in murine adipocytes; these molecules may be intermediates in glucose metabolism. It is unclear why 2-DG-treated cells had lower levels of SCD expression than those in glucose-free conditions. We have measured the cellular ATP concentration under these conditions and found that it was comparable in fructose and 2-DG treatments, and that ATP levels under these treatments were approximately half those found in glucose-treated adipocytes and are comparable with changes reported in other studies [9].

In conclusion, we have demonstrated that SCD, a key gene in lipid metabolism, is directly regulated by glucose availability in adipose cells. Coupled with our report describing regulation of SCD expression by PUFAs in 3T3-L1 adipocytes [6], these findings collectively demonstrate that the SCD gene is sensitive to the nutritional status of the adipocyte. The specific metabolite(s) of glucose responsible for SCD regulation in adipocytes needs further investigation, since it appears that SCD may be regulated independently of other glycolytic and lipogenic genes. In addition, the intermediates and mechanisms responsible for SCD regulation in adipocytes also differ from those in liver. Therefore the SCD gene appears to represent a novel model which can be used to provide additional insight into the mechanisms of nutritional control of the genes of lipid metabolism in adipose cells.

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