Comparison of In Vivo Effects of Insulin on SREBP-1c Activation and INSIG-1/2 in Rat Liver and Human and Rat Adipose Tissue

Guenther Boden[1], **Sajad Salehi**[1], **Peter Cheung**[1], **Carol Homko, R.N.**[1], **Weiwei Song**[1], **Catherine Loveland-Jones**[2], and **Senthil Jayarajan**[2]

[1]Division of Endocrinology/Diabetes/Metabolism and the Clinical Research Center, Temple University School of Medicine, Philadelphia, PA

[2]Department of Surgery, Temple University School of Medicine, Philadelphia, PA

Abstract

The stimulatory effects of insulin on de novo lipogenesis (DNL) in the liver, where it is an important contributor to non-alcoholic fatty liver disease (NAFLD), hepatic and systemic insulin resistance, is strong and well established. In contrast, insulin plays only a minor role in DNL in adipose tissue. The reason why insulin stimulates DNL more in liver than in fat is not known but may be due to differential regulation of the transcription and post-translational activation of sterol regulatory element binding proteins (SREBPs). To test this hypothesis, we have examined effects of insulin on activation of SREBP-1c in liver of rats and in adipose tissue of rats and human subjects. Liver and epidydimal fat were obtained from alert rats and subcutaneous adipose tissue from human subjects in response to 4 h euglycemic-hyperinsulinemic clamps.

Here we show that acutely raising plasma insulin levels in rats and humans increased SREBP-1 mRNA comparably 3-4 fold in rat liver and rat and human adipose tissue, but increased post-translational activation of SREBP-1c only in rat liver, while decreasing it in adipose tissue. These differential effects of insulin on SREBP-1c activation in liver and adipose tissue were associated with robust changes in the opposite direction of INSIG-1 and to a lesser extent of INSIG-2 mRNA and proteins.

We conclude that these findings support the hypothesis that insulin stimulated activation of SREBP-1c in the liver, at least in part, by suppressing INSIG-1 and -2, whereas in adipose tissue, an increase in INSIG-1 and -2 prevented SREBP-1c activation.

INTRODUCTION

A key action of insulin is regulation of de novo lipogenesis (DNL), i.e., the synthesis of fatty acids from excess carbohydrates (CHO) which cannot be oxidized nor stored as glycogen

Correspondence: Guenther Boden, M.D. Temple University Hospital 3401 North Broad Street Philadelphia, PA 19140 Tel: 215-707-8984 Fax: 215-707-1560 bodengh@tuhs.temple.edu.

(1,2). Although DNL has been demonstrated to be active in both liver and adipose tissue (1), levels of DNL activity in these two tissues are different. In the liver, DNL can be responsible for up to 30% of hepatic fat in obese subjects (3), is an important contributor to the development of non-alcoholic fatty liver disease (NAFLD) and hepatic as well as systemic insulin resistance (4). In adipose tissue, however, DNL plays only a minor role and contributes little to adipose tissue triglyceride stores (5,6). The reason why insulin stimulates DNL more in liver than in fat is not known.

In the liver, insulin controls DNL by regulating the transcription and post-translational activation of sterol regulatory element binding proteins (SREBPs) (Reviewed in ref. 7). SREBP-1c, the predominant isoform in liver and adipose tissue (8, 9), is synthesized as a precursor protein and retained in an inactive form in the endoplasmic reticulum (ER), where it is bound to two other proteins, insulin induced gene (INSIG) and SREBP cleavage activating protein (SCAP) (Reviewed in ref. 9). Activation of SREBP-1c requires dissociation from INSIG proteins and translocation of the SREBP/SCAP complex to the Golgi complex, where SREBP-1c undergoes a two-step proteolytic process, liberating the N-terminal, transcriptionally active (~ 68 kD) form of SREBP-1c (9). Whereas the molecular details for this process are not clear, there is evidence for an important role of the INSIG proteins (7, 10-12).

The large difference in insulin stimulation of DNL in liver and adipose tissue (5,6) suggests that there are probably differences in insulin stimulation of SREBP-1c activation between liver and fat. Surprisingly, however, in contrast to a large body of information in liver, there is little information concerning insulin regulation of SREBP-1c activation in adipose tissue. In fact, we are not aware of in vivo data in human or rat adipose tissue of insulin effects on either SREBP-1c activation nor on INSIG-1 or INSIG-2 expression.

The objective of the current study was to explore possible reasons for the different insulin effects on DNL in liver and fat. To this end, we compared acute, in vivo effects of physiological hyperinsulinemia on transcription, translational and post-translational activation of SREBP-1c and on INSIG-1 and INSIG-2 mRNA and proteins in liver and epididymal fat of rats and in subcutaneous fat of human subjects.

METHODS AND PROCEDURES

Male Sprague-Dawley rats (~ 300 g purchased from Charles River Laboratories, Wilmington, MA) were used and procedures were as described (13). Briefly, a polyvinyl catheter was inserted into the internal jugular vein and extended to the right atrium. This catheter was used for infusions. A second catheter was advanced through the left carotid artery to the aortic arch. This catheter was used for blood collections. The rats were allowed one week to recover from the effects of surgery. At that time they were within 3% of their preoperative weight.

Rat Euglycemic-hyperinsulinemic clamp studies

Clamps were performed with awake, unrestrained rats. Insulin (28 pmol/kg min) was infused through the jugular vein catheter for 4 h. Glucose concentrations were clamped at

euglycemic levels by a variable rate infusion of 25% glucose. Blood glucose levels were monitored with an Elite Glucometer (Bayer, Elkhart, IN) and glucose infusion rates (GIR) were adjusted every 5-10 minutes as needed.

Rat Saline Controls

Saline was infused for 4 h, plasma glucose concentrations were maintained at ~ 5.5 mmol/l by a variable rate infusion of glucose.

After the studies, the rats were killed by an overdose of carbon monoxide gas and the livers and the epididymal fat was freeze clamped, excised and kept frozen at -80°C until assayed.

All protocols were approved by the Temple University Institutional Animal Care and Use Committee.

Human Studies

Study subjects (Table 1) were admitted to the Temple University Hospital Clinical Research Center on the day before the studies. At ~ 0800 h on the day of the study, venous blood samples were obtained and an open subcutaneous fat biopsy was performed by a surgeon from the lateral aspect of one upper thigh as described (14). The excised fat (200-300 mg) was dropped immediately into isopentane kept at its freezing point by liquid nitrogen. Following the initial fat biopsy, a euglycemic-hyperinsulinemic clamp was performed. At the end of the clamp, another fat biopsy was obtained from the contralateral leg. The frozen fat was stored at -80°C until analyzed. Informed written consent was obtained from all subjects after explanation of the nature, purpose, and the potential risks of these studies. The study protocol was approved by the Institutional Review Board of Temple University Hospital.

Euglycemic-Hyperinsulinemic Clamping in Humans

To obtain comparable serum insulin levels in humans and rats, regular human insulin was infused IV in humans at a rate of 14 pmol/kg min. Plasma glucose concentrations were clamped at ~ 5.5 mmol/l by a feedback controlled variable glucose infusion. Glucose samples were obtained before and at hourly intervals during insulin infusions for determination of plasma glucose and insulin levels.

Western blots

Liver and adipose tissues were extracted and protein content measured as described (13). Blots were probed with the following antibodies from Santa Cruz Biotechnology (Santa Cruz, CA): INSIG-1 (SC-25124-rat and human), INSIG-2 (SC-66936 rat and human), SREBP-1c (SC-8984 rat), ChREBP (SC-33764), FAS (SC-55580).

Real-time reverse transcriptase PCR

Total RNAs were isolated from frozen rat liver and fat and real-time reverse transcriptase PCR was performed as described (13,14). Rat and human primers are shown in Table 2.

Statistical Analysis

All data are expressed as means ± SE. Statistical analyses were performed using the SigmaStat program (Aspire Software, Ashburn, VA). Group comparisons were made with a one-way ANOVA or the Kruskal-Wallis ANOVA on Ranks when the samples were drawn from a non-normal population or did not have equal variances. Population normality was tested using the Kolmogorov-Smirnov test with Lilliefors' correction, equal variance was tested using the Levene Median test. Post-hoc testing was done with the Student-Newman-Keuls Test.

RESULTS

Glucose, insulin, FFA and GIR during euglycemic-hyperinsulinemic clamping

Glucose concentrations were clamped at $5.5 ± 0.5$ mmol/l during saline and insulin infusions in rats and were $5.2 ± 0.2$ and $5.5 ± 0.1$ mmol/l pre and post insulin, respectively, in human subjects.

Insulin levels were $237 ± 35$ pmol/l during saline and $1659 ± 56$ pmol/l during insulin in rats and $39 ± 11$ and $1501 ± 123$ pmol/l, during saline and insulin, respectively, in humans.

FFA levels were $524 ± 13$ μmol/l during saline and $175 ± 20$ μmol/l during insulin in rats and $433 ± 39$ and $50 ± 9$ μmol/l, during saline and insulin, respectively, in humans.

In response to hyperinsulinemia, GIR increased from 0 to $218 ± 2$ μmol/kg min in rats and from 0 to $46 ± 7$ μmol/kg min in humans.

Insulin stimulated SREBP-1 mRNA similarly in rat liver and in rat and human fat (Figure 1)

Compared to saline, insulin increased SREBP-1 mRNA 4.1-fold ($11.8 ± 2.3$ vs. $2.9 ± 0.3$, p < 0.02) in rat liver, 4.4-fold ($23.2 ± 4.1$ vs. $5.3 ± 0.3$, p < 0.001) in rat epididymal adipose tissue, and 3.1-fold ($2.5 ± 0.7$ vs. $0.84 ± 0.1$, p < 0.001) in human sc adipose tissue.

As the similar insulin stimulation of SREBP-1c transcription did not explain the differences in DNL in liver and adipose tissue, we next examined insulin effects on post-translational activation of SREBP-1 in these tissues.

Insulin increased post-translational activation of SREBP-1c in liver but not in fat (Figure 2)

In rat liver, insulin increased the abundance of the transcriptionally active (68 kD) form of SREBP-1c 3.3-fold ($0.73 ± 0.12$ vs. $0.22 ± 0.01$, p < 0.02) while having no significant effect on the 125 kD precursor form of SREBP-1c resulting in a 3.1-fold increase in the 68/125 kD protein ratio. In contrast, in rat epididymal fat, insulin decreased by > 50% both the 68 kD form of SREBP-1c ($0.32 ± 0.06$ vs. $0.73 ± 0.06$, p < 0.01), and the 125 kD precursor form of SREBP-1c ($0.32 ± 0.14$ vs. $0.63 ± 0.2$, p < 0.01). Unfortunately, we were unable to determine SREBP-1c protein in human adipose tissue due to its low concentration and the scarcity of available biopsy material. Similar problems have been reported by other investigators (6).

INSIG-1 is decreased by insulin in liver and increased in fat (Figure 3)

To investigate possible causes for these differences in insulin induced activation of SREBP-1c in liver and adipose tissue, we examined effects of insulin on INSIG-1 and INSIG-2 both of which are considered to play key roles in post-translational SREBP-1c activation (15,16).

In rat liver, insulin, (compared to saline), reduced INSIG-1 mRNA by > 70% (7.5 ± 1.6 vs. 25.4 ± 3.1, $p < 0.001$) and INSIG-1 protein by > 40% (36 ± 8 vs. 60 ± 1, $p < 0.04$).

In rat adipose tissue, insulin increased INSIG-1 mRNA > 30-fold (85 ± 21 vs. 2.6 ± 0.2, $p < 0.01$) and INSIG-1 protein > 2-fold (1.26 ± 0.26 vs. 0.55 ± 0.01, $p < 0.01$).

In human adipose tissue, insulin increased INSIG-1 mRNA 9-fold (7.8 ± 4.0 vs. 0.9 ± 0.3, $p = 0.05$) and INSIG-1 protein 1.8-fold (1.2 ± 0.2 vs. 2.1 ± 0.5, $p < 0.05$).

Insulin effects on INSIG-2 (Figure 4)

In rat liver, insulin, (compared to saline), reduced INSIG-2 mRNA by > 40% (8.7 ± 1.7 vs. 15.4 ± 2.5, $p < 0.03$) and INSIG-2 protein by > 50% (10.1 ± 2.4 vs. 4.3 ± 19, $p < 0.05$).

In rat adipose tissue, INSIG-2 mRNA did not change in response to insulin (4.5 ± 1.3 vs. 3.8 ± 0.4, NS) but INSIG-2 protein increased 1.8-fold (8.1 ± 0.8 vs. 4.4 ± 1.1, $p < 0.03$).

In human adipose tissue, neither INSIG-2 mRNA nor protein changed significantly in response to insulin (2.4 ± 0.8 vs. 2.6 ± 0.9, NS and 1.0 ± 0.4 vs. 1.8 ± 0.5, NS, respectively).

Effects of insulin on SREBP-1c targets (Figure 5)

An increase in SREBP-1c alone has been reported to be sufficient to promote an increase in the transcription of glucokinase (GK) (17) and to mediate the inhibitory effect of insulin on phosphenolpyruvate (PEPCK) (18). In agreement, we found a > 15-fold increase in GK mRNA and a > 80% suppression of PEPCK mRNA associated with the > 4-fold increase in the transcriptionally active 68 kD form of SREBP-1c. On the other hand, SREBP-1c is known to need collaboration of several other transcription factors, including carbohydrate regulatory element binding protein (ChREBP) (17,19) and X box protein-1 (XBP-1) (20) to promote expression of lipogenic enzymes. In this study, euglycemia-hyperinsulinemia was not associated with significant changes in the transcription factors ChREBP and XBP-1s, nor with changes in mRNAs of the two DNL related enzymes, fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC).

DISCUSSION

Our main objective was to compare in vivo effects of insulin on transcription and post-transcriptional activation of SREBP-1c in rat liver and rat and human adipose tissue. The results showed that acutely raising plasma insulin to postprandial levels, increased SREBP-1 mRNA comparably (3-4-fold) in livers and epidydimal adipose tissue of alert, free moving rats and in s.c. adipose tissue of human subjects. These in vivo findings confirmed previous in vitro reports in rat hepatocytes (15, 16, 21) and further showed that insulin had similar

effects in rat and human adipocytes. They did not explain, however, the different in vivo insulin effects on DNL in liver and fat (5,6).

On the other hand, we also found that insulin action on the post-translational activation of SREBP-1c protein was distinctly different in liver and adipose tissue. In rat liver, physiologic hyperinsulinemia produced a > 3-fold increase in the activated 68 kD SREBP-1c protein indicating strong post-translational activation and confirming previous reports in hepatocytes albeit obtained with superphysiological (100 nM) insulin doses (12,22). In contrast, to livers in rat adipose tissue, the 68 kD SREBP-1c protein did not increase. This failure of physiological insulin doses to activate SREBP-1c in viv*o* in adipose tissue has not previously been reported and may be one reason why in fat insulin stimulation of DNL has been found to be relatively weak (5,6).

To explore the reason why insulin activated SREBP-1c protein in the liver but not in adipose tissue, we determined INSIG-1/2 responses to insulin. Both INSIG-1 and INSIG-2 have been shown to bind SREBP-1c and to prevent the ER to Golgi transfer of the SREBP-1c/ SCAP complex and the proteolytic activation of SREBP-1c (Reviewed in ref. 23). Here, we found that in rat liver, the hyperinsulinemia associated activation of SREBP-1c protein was accompanied by downregulation of both INSIG-1 and INSIG-2 mRNAs and proteins. In contrast, in rat as well as in human adipose tissue, the lack in SREBP-1c activation was accompanied by a strong increase in INSIG-1 mRNA and proteins. INSIG-2 protein also increased in rat fat but less than INSIG-1 and neither INSIG-2 mRNA or protein changed in human fat.

Our findings in rat liver confirm reports in hepatocytes showing that activation of SREBP-1c was associated with insulin reduced levels of INSIG-2 mRNA and protein (12, 24). The are also in agreement with reports showing that hepatic overexpression of INSIG-1 (or INSIG-2) inhibited activation of SREBP-1c in rat liver (11, 25, 26) and that lowering of INSIG-1 in Chinese hamster ovary cells increased proteolytic activation of SREBP-1c, whereas overexpression of INSIG-1 decreased SREBP-1c activation (27). On the other hand, our findings seem to disagree with results from a study that showed that INSIG-1 mRNA was decreased in livers of fasted rats and increased back to basal levels during refeeding (24). The reason for this apparent discrepancy, while not entirely clear, maybe related to differences in the experimental protocol. Yabe et al. (24) studied livers from mice after oral feeding, whereas we studied livers of rats after 4 h of IV insulin infusions. Thus, oral food ingestion and IV insulin administration may have resulted not only in different portal venous insulin concentrations, but oral feeding was also likely to have induced release of gastrointestinal factors which could have influenced INSIG-1 expression.

With respect to INSIG responses to insulin in adipocytes or fat, we found only one report (28) which showed strong increase in INSIG-1 mRNA in adipocytes of rats at the onset of high fat diet induced obesity who presumably, were hyperinsulinemic.

Taken together, our findings are compatible with the notion that the insulin mediated activation of SREBP-1c in the liver was caused, at least in part, by the downregulation of

INSIG-1 and to a lesser extent INSIG-2, while the lack of SREBP-1c activation in adipose tissue was due to upregulation of INSIG-1 and to a lesser extent INSIG-2.

Clearly, our findings do not prove causality nor can they exclude other causes. For instance, endoplasmic reticulum (ER) stress has been found to be associated with activation of SREBPs in various conditions and cell types including livers of genetic models of obesity (ob/ob mice, Zucker fa/fa rats) (29), homocysteine induced ER stress (30), ethanol fed rodents (31) and various cell types under ER stress (29, 32, 33). The linkage of ER stress to SREBP activation is an attractive concept as both the activation of ATF-6, one of the ER stress proximal sensors, and SREBP-1 are induced by the same proteases and in the same locations (32). In support of this concept, we have previously shown that 4 hours of euglycemic-hyperinsulinemia produced a modest degree of ER stress in rat liver (34). It remains to be shown, however, whether insulin induced ER stress is also present in adipose tissue and if so, how it compares to ER stress in liver.

Insulin, via SREBP-1c, activates expression of many genes involved in DNL (9). We, have, therefore, examined in rat liver effects of insulin on several SREBP-1c target genes including glucokinase (GK), the key enzyme in hepatic glucose uptake/phosphorylation, phosphenolpyruvate carboxykinase (PEPCK), a key gluconeogenetic enzyme, acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS), two sequential enzymes in the DNL pathway. The observed insulin induced increase in the active form of SREBP-1c protein in the liver was associated with a > 15-fold increase in GK mRNA and a > 80% decrease in PEPCK mRNA. These *in vivo* results expanded previous data in cultured hepatocytes, which had shown that insulin induced activation of SREBP-1c potently increases GK gene expression (15) and strongly inhibited PEPCK gene expression (18). We found, however, no effect of insulin on ACC nor on FAS mRNA. Perhaps, our 4 h insulin infusions were too short to increase expression of these enzymes. On the other hand, activation of these genes has been shown to depend not only on hyperinsulinemia and activation of SREBP-1c and other transcription factors (35), but also on hyperglycemia (17,19). The absence of hyperglycemia in our studies, therefore, may have prevented the upregulation of ACC and FAS. Supporting this notion, there was no effect of insulin on ChREBP, a transcription factor which has emerged as a major mediator of glucose action on lipogenic gene expression and lipid synthesis (19), nor on XBP-1s, a transcription factor, which has recently been shown to be induced by CHO feeding and to be required for hepatic DNL (20). Thus, our findings are in accord with the concept that stimulation of DNL in the liver requires an excess of carbohydrates that need to be stored as fat in addition to insulin induced activation of SREBP-1c.

In summary, our results showed that acutely raising plasma insulin levels in rats and humans increased SREBP-1 mRNA comparably in rat liver and rat and human adipose tissue, but increased post-translational activation of SREBP-1c only in rat liver, while decreasing it in adipose tissue. These differential effects of insulin on SREBP-1c activation in liver and adipose tissue were associated with changes in the opposite direction of INSIG proteins. Thus, the insulin induced activation of SREBP-1c in the liver was accompanied by a decrease in INSIG-1 and INSIG-2 protein, whereas in adipose tissue, the insulin induced decrease in SREBP-1c activation was accompanied by an increase in INSIG-1 and INSIG-2

proteins. These findings support the hypothesis that insulin stimulated activation of SREBP-1c in the liver, at least in part, by suppressing INSIG-1 and -2, whereas in adipose tissue, an increase in INSIG-1 and -2 prevented SREBP-1c activation.

ACKNOWLEDGEMENTS

REFERENCES

1. Shargo E, Glennon J, Gord E. Comparative aspects of lipogenesis in mammalian tissues. Metabolism. 1971; 20:54–62. [PubMed: 4992541]

2. Angel A, Bray G. Synthesis of fatty acids and cholesterol by liver, adipose tissue and intestinal mucosa from obese and control patients. Eur J Clin Invest. 1979; 9:355–362. [PubMed: 118029]

3. Donnelly KL, Smith CJ, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest. 2005; 115:1343–1351. [PubMed: 15864352]

4. Cohen JC, Horton JD, Hobbs HH. Human fatty liver disease: Old questions and new insights. Science. 2011; 332:1519–1523. [PubMed: 21700865]

5. Diraison F, Yankah V, Letexier D, Dusserre E, Jones P, Beylot M. Differences in the regulation of adipose tissue and liver lipogenesis by carbohydrates in humans. J Lipid Research. 2003; 44:846–853. [PubMed: 12562844]

6. Letexier D, Pinteur C, Large V, Frering V, Beylot M. Comparison of the expression and activity of the lipogenic pathway in human and rat adipose tissue. J Lipid Res. 2003; 44:2127–2134. [PubMed: 12897191]

7. Raghow R, Yellaturu C, Deng X, Park EA, Elam MB. SREBPs: The crossroads of physiological and pathological lipid homeostasis. Cell. 2007; 19:65–73.

8. Shimomura I, Shimano H, Horton JD, Goldstein JL, Brown MS. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. J Clin Invest. 1997; 99:838–845. [PubMed: 9062340]

9. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest. 2002; 109:1125–1131. [PubMed: 11994399]

10. Yabe D, Brown MS, Goldstein JL. Insig-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. PNAS. 2002; 99:12753–12758. [PubMed: 12242332]

11. Takaishi K, Duplomb L, Wang M-Y, Li J, Unger RH. Hepatic insig-1 or -2 overexpression reduces lipogenesis in obese Zucker diabetic fatty rats and in fasted/refed normal rats. PNAS. 2004; 101:7106–7111. [PubMed: 15096598]

12. Yellaturu CR, Deng X, Park EA, Raghow R, Elam MB. Insulin enhances the biogenesis of nuclear sterol regulatory element-binding protein (SREBP)-1c by posttranscriptional down-regulation of Insig-2A and its dissociation from SREBP cleavage-activating protein (SCAP)-SREBP-1c complex. J Biol Chem. 2009; 284:31726–31734. [PubMed: 19759400]

13. Boden G, She P, Mozzoli M, Cheung P, Gumireddy K, Reddy P, Xiang X, Ruderman N. Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor-κB pathway in rat liver. Diabetes. 2005; 54:3458–3465. [PubMed: 16306362]

14. Boden G, Silviera M, Smith B, Cheung P, Homko C. Acute tissue injury caused by subcutaneous fat biopsies produces endoplasmic reticulum stress. J Clin Endocrinol. 2010; 95:349–352.

15. Foretz M, Guichard C, Ferre P, Foufelle F. Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. PNAS. 1999; 96:12737–12742. [PubMed: 10535992]

16. Shimomura I, Bashmakov Y, Ikemoto S, Horton JD, Brown MS, Goldstein JL. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. Proc Natl Acad Sci USA. 96:13656–13661. 199. [PubMed: 10570128]

17. Foufelle F, Girard J, Ferre P. Regulation of lipogenic enzyme expression by glucose in liver and adipose tissue: A review of the potential cellular and molecular mechanisms. Advan Enzyme Regul. 1996; 36:199–226. [PubMed: 8869748]

18. Chakravarty K, Leahy P, Becard D, Hakimi P, Foretz M, Ferre P, Foufelle F, Hanson RW. Sterol regulatory element-binding protein-1c mimics the negative effect of insulin on phosphoenolpyruvate carboxykinase (GTP) gene transcription. J Biol Chem. 2001; 276:34816–34823. [PubMed: 11443121]

19. Postic C, Dentin R, Denechaud PD, Girard D. ChREBP, a transcriptional regulator of glucose and lipid metabolism. Ann Rev Nutrition. 2007; 27:179–192. [PubMed: 17428181]

20. Lee A-H, Scapa EF, Cohen DE, Glimcher LH. Regulation of hepatic lipogenesis by the transcription factor XBP1. Science. 2008; 320:1492–1496. [PubMed: 18556558]

21. Azzoult-Marniche D, Becard D, Guichard C, Foretz M, Ferre P, Foufelle F. Insulin effects on sterol regulatory-element-binding protein-1c (SREBP-1c) transcriptional activity in rat hepatocytes. Biochem J. 2000; 350:389–393. [PubMed: 10947952]

22. Yellaturu CR, Deng X, Cagen LM, Wilcox HG, Park EA, Raghow R, Elam MB. Post-translational processing of SREBP-1 in rat hepatocytes is regulated by insulin and cAMP. Biochem Biophys Research Commun. 2005; 332:174–180. [PubMed: 15896314]

23. Xiao-Ying D, Sheng-Qui T. Insulin-induced gene: A new regulator in lipid metabolism. Peptides. 2012; 31:2145–2150.

24. Yabe D, Komura R, Liang G, Goldstein JL, Brown MS. Liver-specific mRNA for Insig-2 down-regulated by insulin: Implications for fatty acid synthesis. PNAS. 2003; 100:3155–3160. [PubMed: 12624180]

25. Engelking LJ, Kuriyama H, Hammer RE, et al. Overexpression of Insig-1 in the livers of transgenic mice inhibits SREBP processing and reduces insulin-stimulated lipogenesis. J Clin Invest. 2004; 113:1168–1175. [PubMed: 15085196]

26. Yang T, Espenshade PJ, Wright WE, et al. Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. Cell. 2002; 110:489–500. [PubMed: 12202038]

27. Lee JN, Ye J. Proteolytic activation of sterol regulatory element-binding protein induced by cellular stress through depletion of Insig-1. J Biol Chem. 2004; 279:45257–45265. [PubMed: 15304479]

28. Li J, Yu X, Pan W, Unger RH. Gene expression profile of rat adipose tissue at the onset of high-fat-diet obesity. Am J Physiol Endocrinol Metab. 2002; 282:E1334–E1341. [PubMed: 12006364]

29. Kammoun HL, Chabanon H, Hainault I, et al. GRP78 expression inhibits insulin and ER stress-induced SREBP-1c activation and reduces hepatic steatosis in mice. J Clin Invest. 2009; 119:1201–1215. [PubMed: 19363290]

30. Westruck GH, Lentz SR, Dayal S, et al. Homocyteine-induced endoplasmic reticulum stress causes dysregulation of the cholesterol and triglyceride biosynthetic pathways. J Clin Invest. 2001; 107:1263–1273. [PubMed: 11375416]

31. Ji C, Chan C, Kaplowitz N. Predominant role of sterol response element binding proteins (SREBP) lipogenic pathways in ehpatic steatosis in the murine intragasric ethanol feeding model. J Hepatol. 2006; 45:717–724. [PubMed: 16879892]

32. Ye J, Rawson RB, Komuro R, et al. ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. Mol Cell. 2000; 6:1355–1364. [PubMed: 11163209]

33. Bobrovinikova-Marjon E, et al. PERK-dependent regulation of lipogenesis during mouse mammary gland development and adipocyte differentiation. Proc Nat Acad Sci. 2008; 105:16314–16319. [PubMed: 18852460]

34. Boden G, Song W, Duan X, et al. Infusion of glucose and lipids at physiological rates causes acute endoplasmic reticulum stress in rat liver. Obesity. 2011; 19:1366–1372. [PubMed: 21475143]

35. Deng X, Yellaturu C, Cagen L, Wilcox HG, Park EA, Raghow R, Elam MB. Expression of the rat sterol regulatory element-binding protein-1c gene in response to insulin is mediated by increase transactivating capacity of specificity protein (Sp1). J Biol Chem. 2002; 282:17517–17529. [PubMed: 17449871]
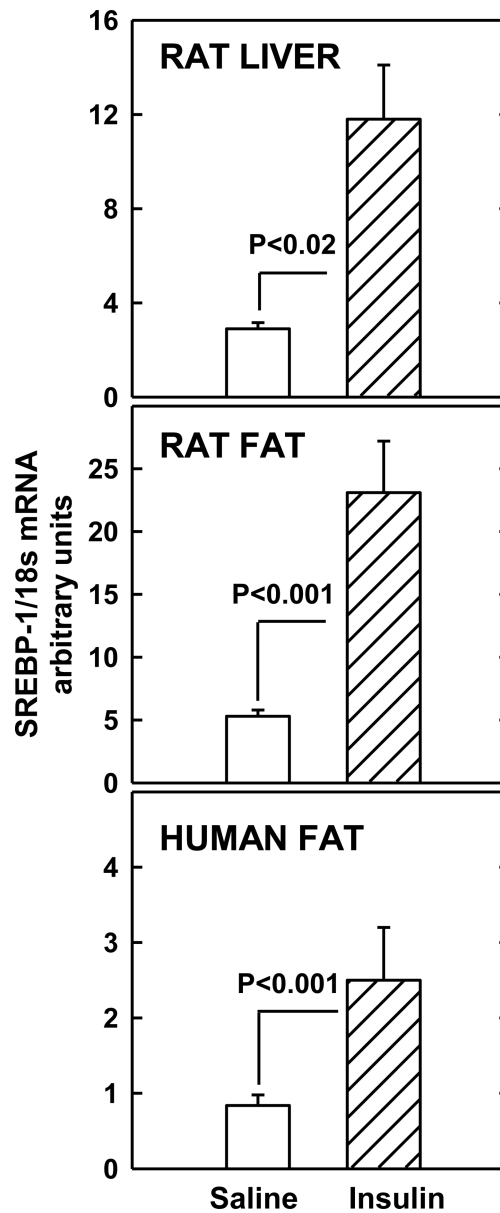
Figure 1.
Effects of 4 h infusions of saline or insulin (euglycemic-hyperinsulinemia) on SREBP-1 mRNA in rat liver, rat epididymal adipose tissue (RAT FAT) and human subcutaneous adipose tissue (HUMAN FAT). Rat studies: Saline studies: (n = 4). Insulin studies: (n = 5). Human studies: (n = 6). Saline refers to biopsies taken before, insulin refers to biopsies taken after insulin infusions. Shown are means ± SE.
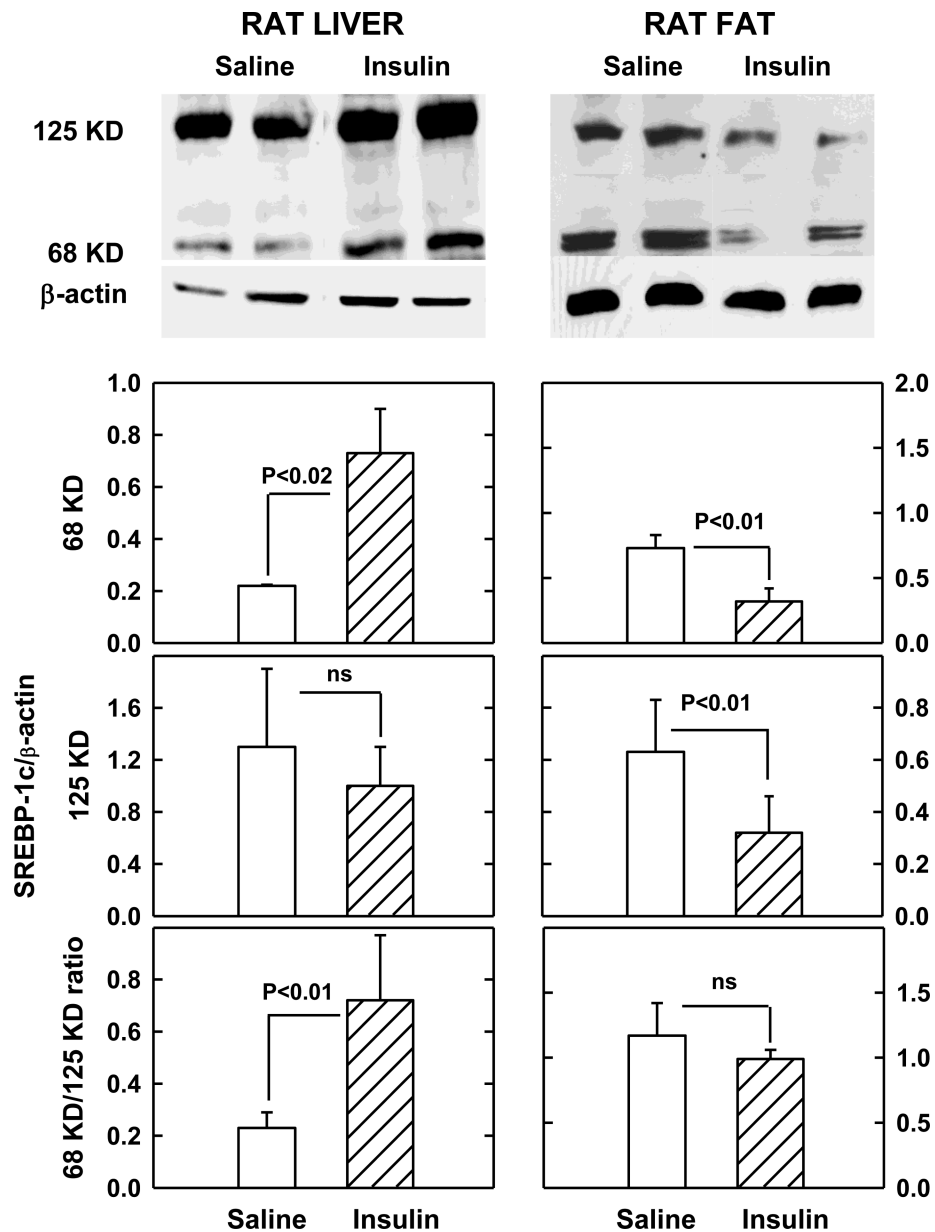
Figure 2.
Top panels: representative Western blots showing effects of 4 h infusions of saline or insulin (euglycemic-hyperinsulinemia) on the post-translational activated (~ 68 kD) and the precursor (~ 125 kD) SREBP-1c protein in rat liver and epididymal adipose tissue (RAT FAT). Lower panels: 68 kD, 125 kD and 68 kD/125 kD SREBP-1c results from 4 rat saline studies and from 5 rat insulin studies. Shown are mean ± SE.
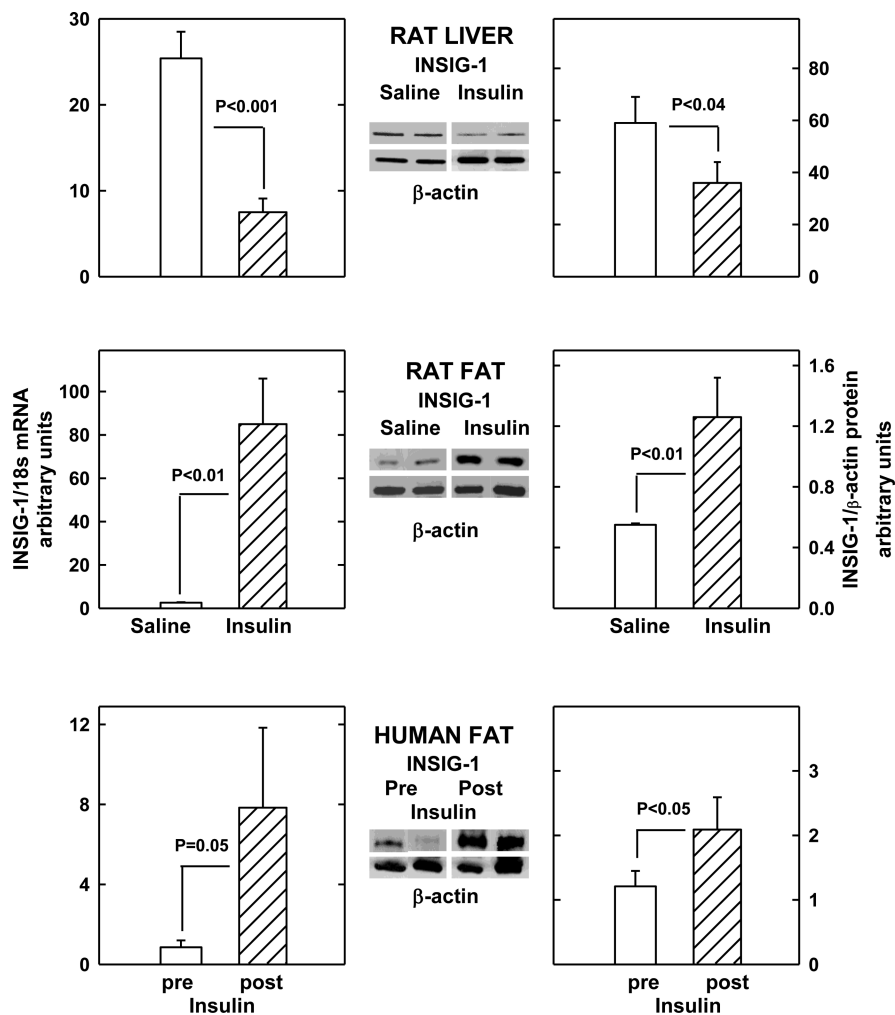
Figure 3.
Upper panels – Effects of 4 h infusions of saline or insulin (euglycemic-hyperinsulinemia) on INSIG-1 mRNA (left), on 2 representative INSIG-1 Western blots (middle) and on INSIG-1 protein (right) in rat liver and epididymal fat (Saline, n = 4, insulin, n = 5). Lower panels – INSIG-1 mRNA (left) 2 representative INSIG-1 Western blots (middle) and INSIG-1 protein (right) before (pre) and after (post) insulin infusions (euglycemic-hyperinsulinemia) from 6 human insulin studies. Shown are mean ± SE.
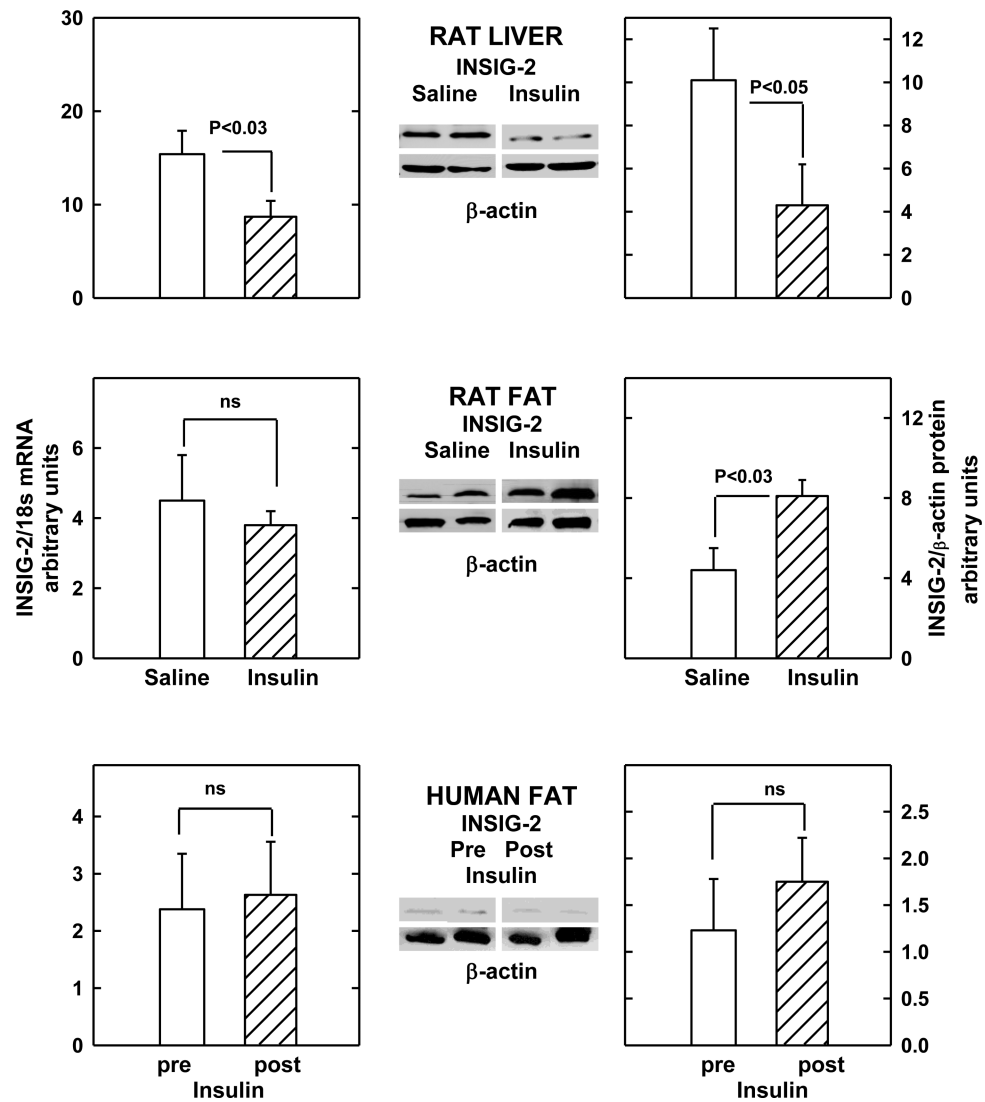
Figure 4.
Upper panels – Effects of 4 h infusions of saline or insulin (euglycemic-hyperinsulinemia) on INSIG-2 mRNA (left), 2 representative INSIG-2 Western blots (middle) and INSIG-2 protein (right) in rat liver and epididymal fat. (Saline, n = 4, insulin, n = 5).
Lower panels: INSIG-2 mRNA (left), 2 representative INSIG-2 Western blots (middle) and INSIG-2 protein (right) before (pre) and after (post) insulin infusions (euglycemia-hyperinsulinemia) from 6 human studies. Shown are mean ± SE.
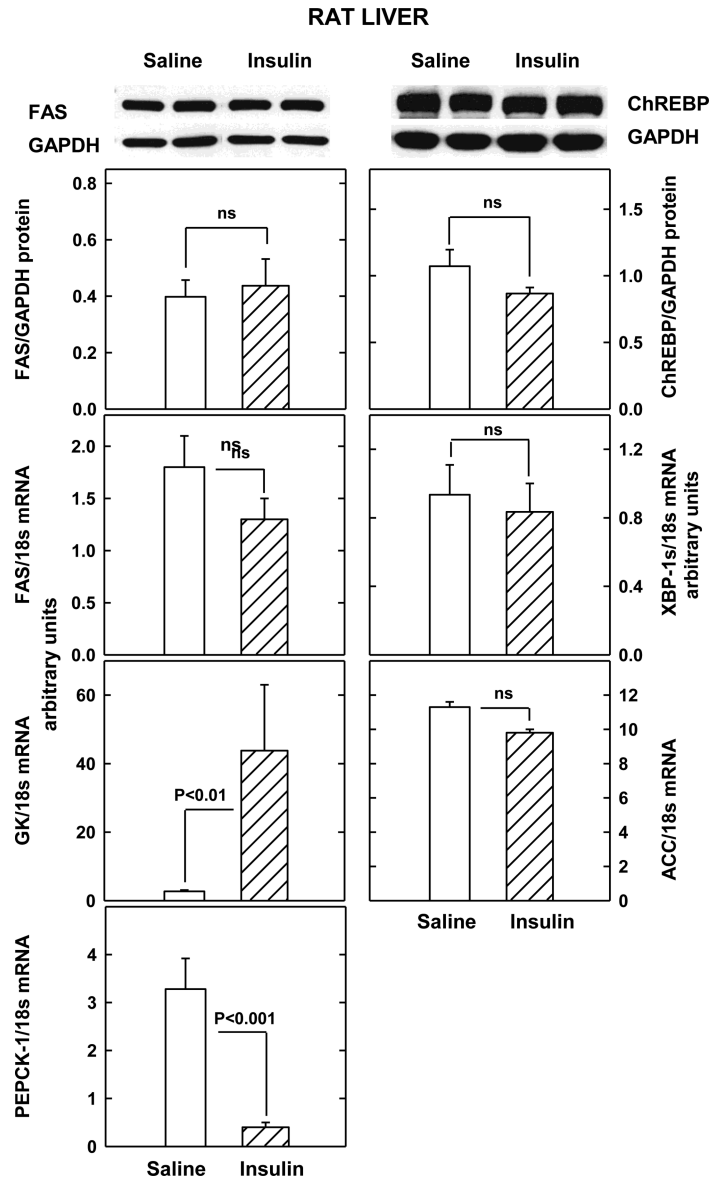
Figure 5.
Effects of 4 h saline or insulin (euglycemic-hyperinsulinemia) infusions on XBOX Protein 1 s (XBP1s) mRNA, glucokinase (GK) mRNA, phosphoenolpyruvate carboxykinase (PEPCK) mRNA, carbohydrate response element binding protein (ChREBP) protein, fatty acid synthase (FAS) mRNA and acetyl-CoA carboxylase (ACC) mRNA in rat liver. Also shown are representative ChREBP Western blots. Saline studies n = 4, insulin studies n = 5. Shown are mean ± SE.

Table 1

Study subjects

	Mean (n = 7)	SEM
Age yr	42	6
Gender	4M/2F	
Ht cm	176.8	5.2
Wt kg	87.673	2.543
BMI kg/m2	28.72	1.09

Table 2

		Rat	Human
ACC	forward primer	gaatggctggagaagcagct	
	reverse primer	tacaacctctgctcgctgag	
FAS	forward primer	caaacctggctgcctactac	
	reverse primer	ggagcacatctcgaaggcta	
INSIG1	forward primer	tgcagatccagcggaatgt	ggcagcttcccaagtattcg
	reverse primer	ccaggcggaggagaagatg	agcaccatcaacctacctcct
INSIG2	forward primer	gacggatgtgttgaaggatttct	caccagatgtgatcaccagc
	reverse primer	tggactgaagcagaccaatgtc	tgtggctctcctagatgcct
PEPCK-1	forward primer	acagagaacaggctagggac	
	reverse primer	ctggattcctgagtgacctc	
SREBP1	forward primer	ggagccatggattgcacatt	tgctgacggaagccaacttg
	reverse primer	aggaaggcttccagagagga	aagtagcacaggagcctcag
ChREEP	forward primer	gtccgatatctccgacacactctt	
	reverse primer	cattgccaacataagcgtcttctg	
XBP1S	forward primer	ttgagaaccaggagttaa	
	reverse primer	cctgcacctgctgcggact	
18S		Ambion catalog # 5103G	

The SREBP primers do not differentiate between SREBP-la and 1c. However, in human and mouse tissues, SREBP-1c is the predominant isoform with 1c to 1a ratios ranging from 10:1 in liver and 3:1 in adipose tissue (13).