0013-7227/99/$03.00/0
Endocrinology

Vol. 140, No. 7
Printed in U.S.A.

Editorial: Nutrition and Fat Cell Differentiation

Over the past decade, there has been an explosion of knowledge about obesity and a great focus on the complex program involved in fat cell differentiation. The temporally orchestrated dance between transcription factors that leads to lipid loading and enhanced insulin sensitivity is fascinating and has increased our understanding of cell fate determination for adipocytes, as well as serving as a model for understanding other cellular differentiation programs (1). It is believed that elucidation of adipocyte biology will facilitate development of strategies to combat obesity, a major problem in the U.S. and worldwide. Additionally, because increased adiposity is associated with insulin resistance and type 2 diabetes, the fat cell could be the key to unlocking the pathophysiology of those diseases as well. The article by Wang *et al.* in this month's journal demonstrates the importance of understanding normal adipocyte biology as a backdrop for the examination of pathophysiology (2). The authors demonstrate that metabolic signals (glucose and insulin) in excess are capable of down-regulating CCAAT enhancer binding protein α (C/EBPα), a transcription factor essential for maintenance of a fully differentiated adipocyte phenotype (2–4). This is a critical observation because loss of complete differentiation correlates with increased insulin resistance and leads to a cavalcade of metabolic derangements. It also provides some insight into the mechanism whereby fat cell differentiation agents, peroxisome proliferator activated receptor γ (PPARγ) agonists, lead to improvement in insulin sensitivity in the setting of hyperglycemia.

Adipocyte Differentiation Program

Many reviews have been written about adipocyte differentiation (1, 5). Recently, it has been observed by two groups that a positive feedback loop occurs between C/EBPα and PPARγ that is responsible for maintenance of terminal differentiation (3, 4). If C/EBPα or PPARγ are disrupted by molecular strategies before adipocyte differentiation, cells will not reach maturity or become insulin sensitive in terms of glucose transport. Many of the genes essential for maintenance of a well differentiated adipocyte phenotype require C/EBPα including: insulin receptor, insulin receptor substrate-1, fatty acid synthase, fatty acid binding protein, and PPARγ (5, 6). C/EBPα also regulates glucose transporter 4 (GLUT-4) by a more complex mechanism (7, 8). This can be reversed by heterodimer formation with the inactive form of C/EBPβ. It has been suggested that loss of C/EBPα could account for adipocyte immaturity and insulin resistance. In most instances, the proposed model has been incomplete differentiation of adipocytes in culture (3, 4). The report by Wang *et al.* (2) suggests a mechanism whereby an abnormal metabolic environment (high glucose in the presence of insulin) can affect mature adipocytes leading to partial differentiation to an insulin-resistant, immature adipocyte (2).

Adipocyte Differentiation and Insulin Sensitivity

During adipocyte differentiation, immature adipocytes are exposed to agents that induce a series of transcription factors in a specific sequence. Initially, C/EBPβ is induced, which induces PPARγ and subsequently C/EBPα (1, 5, 9, 10). C/EBPα and PPARγ positively regulate each other's expression, and this feedback loop maintains fat cell phenotype (3–5). One critical characteristic of mature adipocyte phenotype is the induction of GLUT-4 and distribution of glucose transporters to insulin-responsive vesicles. Only when transporters are expressed and appropriately localized will insulin sensitivity occur. Expression and redistribution of GLUT-4 does not occur in C/EBPα deficient cell lines (3, 4). C/EBPα is necessary for GLUT-4 transcription (11, 12). It has been demonstrated *in vitro* with correlative studies *in vivo* that cytokines such as tumor necrosis factor (TNF)α can induce insulin resistance in adipocytes and that at least one mechanism of this resistance is a decrease in GLUT-4 content. TNFα appears to act by permitting C/EBPβ to enter the nucleus and heterodimerize with C/EBPα or to form C/EBPβ homodimers (8). In this model, GLUT-4 expression is regulated by C/EBPβ translocation rather than C/EBPα content. Regardless of the mechanism, TNFα is able to induce insulin resistance and an effect that can be reversed by TNFα antagonists. Interestingly, adipocytes produce TNFα. One mechanism whereby increased adiposity leads to insulin resistance is postulated to be fat cell production of TNFα.

Insulin Sensitizers and Fat Cell Differentiation

Given this correlation between fat cell mass and TNFα, it has been intriguing to watch the development of the thiazolidinedione class of antidiabetic agents and to unravel the relationship between adipocyte differentiation and whole animal insulin sensitivity. From a cell biology perspective, it is well established that adipocytes do not become insulin sensitive to glucose transport until late in the differentiation program (3, 4). So, it makes sense that agents capable of increasing adipocyte differentiation would improve insulin sensitivity. On the other hand, adipocyte differentiating agents will recruit new adipocytes. This should lead to increased adiposity and insulin resistance. The data presented by Wang *et al.* suggest that derangements in nutrient availability can alter a critical protein essential for the maintenance of adipocyte differentiation (2). If that is the case, this could lead to insulin resistance and a differentiating agent, such as a thiozolidinedione, could restore differentiation and thus enhance insulin sensitivity. Perhaps we should reframe our view of diabetes and insulin resistance as a consequence

Received April 26, 1999.
Address all correspondence and requests for reprints to: Jane E.-B. Reusch, Denver VA Medical Center, 1055 Clermont Street, Denver Colorado 80220. E-mail: Reuschi@den-res.org.

of excess adipocity and consider instead a state of altered adipocyte differentiation.

The induction of insulin resistance as a consequence of metabolic fuel derangements (glucose and free fatty acids) is an established phenomenon in animal and human models of insulin resistance and diabetes. Wang *et al.* present data *in vitro* and *in vivo* that a decline in C/EBPα, a primary determinant of adipocyte phenotype, occurs under metabolic conditions common to insulin resistance and diabetes (2). This could be responsible for the decrease in insulin receptor and insulin receptor substrate-1 that has been noted in animal models of insulin resistance and hyperglycemia as both of these proteins are regulated by C/EBPα.

Transcription Factors and Adipocyte Health

As detailed earlier, C/EBPα and PPARγ form a positive feedback loop that maintains adipocyte differentiation (3, 4). Each transcription factor positively affects the expression of the other, either directly or indirectly. A number of laboratories have demonstrated that phosphorylation of PPARγ alters its transactivating potential and leads to insulin resistance (13). Hyperglycemia and intracellular reactive oxygen species can activate MAP kinase and stimulate PPARγ phosphorylation. Similarly, the authors of the paper in this month's journal correlate diminution of C/EBPα with insulin resistance. They demonstrate a mechanism whereby nutrient stress down-regulates C/EBPα, the other key regulator of adipocyte insulin sensitivity (2). Down-regulation of C/EBPα content or PPARγ function could be expected to impact the other transcription factor. This expectation is fulfilled in the *in vivo* studies undertaken by Wang *et al.* (Fig. 6) (2).

C/EBPα and Cellular Proliferation

One of the main motivations behind the explosion of new information regarding adipocyte biology is a desire to understand obesity and regulation of fat stores. The hope was that understanding adipocyte development would foster strategies for prevention of fat cell accumulation and the related medical consequences. As the story of adipocyte differentiation emerged and the sequential induction of C/EBPβ followed by PPARγ and later C/EBPα, questions arose as to the sufficiency and necessity of each of these events for differentiation. To test the importance of each of these transcription factors for differentiation, molecular tools to introduce either active or dominant negative forms of these molecules were created. In the case of C/EBPα, a large methodological problem arose. Stable cell lines were difficult to create because C/EBPα blocked mitosis by the induction of growth arrest and DNA damage inducible protein 153 and DNA-protein kinase interacting protein 21 (14) leading to cell cycle arrest. C/EBPα arrest of cell cycle is considered a key step in adipocyte terminal differentiation. Down-regulation of C/EBPα could present an opportunity for release into cell cycle and adipocyte proliferation. Theoretically, insulin resistance should not be associated with weight gain as the ability to store fuel is impaired. Despite that logic, insulin resistance and obesity go hand in hand. Perhaps, C/EBPα down-regulation is a permissive step for adipocyte proliferation. Thus, the response of an adipocyte to excess fuel is to develop both insulin resistance to glucose transport and proliferative capacity.

C/EBPα and Whole Animal Physiology

As anticipated from observations with adipocytes in culture, Wang *et al.* (2) find a tight correlation between down-regulation of C/EBPα and decreased PPARγ content. Somewhat unexpected is the fat depot specific down-regulation in epididymal fat pad with a relative sparing of the omental fat pad. Visceral fat, represented in this paper by omental fat, has been shown to correlate with insulin resistance, diabetes, and mortality from atherosclerotic disease (1). Perigonadal fat, represented in this paper by epididymal fat, is also a hormonally active fat depot, but studies have not clearly delineated a relationship between this fat depot and insulin resistance or cardiovascular risk. For the purpose of this discussion, we will treat this epididymal fat depot as representative of peripheral as opposed to central adiposity, which may not be accurate. With the fat pads defined in this way, there is a clear difference in the regional fat pads with regards to the induction of an insulin resistant phenotype. This would be expected to be associated with a more mild insulin resistance than if the visceral fat pad were affected. It would also suggest that the peripheral fat pad would be the target for the PPARγ agonist type of differentiating agents. Thiazolidinediones, in animal models, increase peripheral adipose mass with a relative sparing of visceral adipose stores (Smith, S., SmithKline Beecham-United Kingdom, personal communication). This is not to suggest that the omental fat pad is completely spared, indeed the combination of insulin and glucosamine clearly decreases PPARγ and appears to decrease C/EBPα in Fig. 6 of the article by Wang *et al.* in this issue of *Endocrinology* (2). The critical point to be made by these distinctions is that different fat depots respond differently to metabolic stress and thus could be expected to respond differently to hormonal or pharmaceutical manipulations.

C/EBPα and Nutrition

There is no dispute that there is a relationship between excessive nutrition and adiposity. What has remained more oblique is the relationship between insulin resistance, fat cell differentiation, and the tendency for insulin-resistant animals and humans to gain adipose mass. The dual function of C/EBPα as an inhibitor of cellular proliferation and a key determinant of terminal fat cell differentiation places this transcription factor in a unique position to have important pathophysiological consequences if disregulated. The paper by Wang *et al.* supports this as one mechanism whereby hyperglycemia induces insulin resistance (2).

Future Directions

Much has been learned recently about glucose regulation of transcription, and defining the specific mechanism of this hyperglycemia-related decrease in transcription of C/EBPα could provide a target for pharmaceutical intervention. The precise mechanism of glucose-induced C/EBPα down-reg-

ulation remains to be defined. The data demonstrating an impact of glucosamine and inhibition of the effect in the face of GFAT inhibitors directs one's attention to the glucosamine pathway. Evaluation of the C/EBPα promoter and defining glucose regulatory elements is a clear area for future study. The manuscript by Wang *et al.* provides an exciting lesson on how understanding of normal adipocyte differentiation permits meaningful interpretation of this pathological response to nutrient excess.

Jane E.-B. Reusch
Denver VA Medical Center
Denver, Colorado 80220

and

Dwight J. Klemm
National Jewish Hospital
Denver, Colorado 80206

References

1. **Mandrup S, Lane MD** 1997 Regulating adipogenesis. J Biol Chem 272:5367–5370
2. **Wang Y, Lee-Kwon W, Martindale JL, Adams L, Heller P, Egan JM, Bernier M** 1999 Modulation of CCAAT/enhancer-binding protein-α gene expression by metabolic signals in rodent adipocytes. Endocrinology 140:2938–2947
3. **El-Jack AK, Hamm JK, Pilch PF, Farmer SR** 1999 Reconstitution of insulin-sensitive glucose transport in fibroblasts requires expression of both PPARγ and C/EBPα. J Biol Chem 274:7946–7951
4. **Wu Z, Rosen ED, Brun R, Hauser S, Adelmant G, Troy AE, McKeon C, Darlington GJ, Spiegelman BM** 1999 Cross-regulation of C/EBPα and PPARγ controls the transcriptional pathway of adipogenesis and insulin sensitivity. Mol Cell 3:151–158
5. **Yeh W-C, Cao Z, Classon M, McKnight SL** 1995 Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. Genes Dev 9:168–181
6. **Webster NJ, Kong Y, Cameron KE, Resnik JL** 1994 An upstream element from the human insulin receptor gene promoter contains binding sites for C/EBPβ and NF-1. Diabetes 43:305–312
7. **Kaestner KH, Christy RJ, Lane MD** 1990 Mouse insulin-responsive glucose transporter gene: Characterization of the gene and trans-activation by the CCAAT/enhancer binding protein. Proc Natl Acad Sci USA 87:251–255
8. **Jain R, Police S, Phelps K, Pekala PH** 1999 Tumour necrosis factor-α regulates expression of the CCAAT-enhancer-binding proteins (C/EBPs) α and β and determines the occupation of the C/EBP site in the promoter of the insulin-responsive glucose-transporter gene in 3T3–L1 adipocytes. Biochem J 338:737–743
9. **Gauthier B, Robb M, McPherson R** 1999 Cholesteryl ester transfer protein gene expression during differentiation of human preadipocytes to adipocytes in primary culture. Atherosclerosis 142:301–307
10. **Jiang M-S, Tang Q-Q, McLenithan J, Geiman D, Shillinglaw W, Henzel WJ, Lane MD** 1998 Derepression of the C/EBPα gene during adipogenesis: Identification of AP-2α as a repressor. Proc Natl Acad Sci USA 95:3467–3471
11. **Tang Q-Q, Jiang M-S, Lane MD** Repression of transcription mediated by dual elements in the CCAAT/enhancer binding protein α gene. Proc Natl Acad Sci USA 94:13571–13575
12. **Cooke DW, Lane MD** 1998 A sequence element in the GLUT4 gene that mediates repression by insulin. J Biol Chem 273:6210–6217
13. **Camp HS, Tafuri SR, Leff T** 1999 c-Jun N-terminal kinase phosphorylates peroxisomeproliferator-activated receptor-γ1 and negatively regulates its transcriptional activity.Endocrinology 140:392–397
14. **Timchenko NA, Harris TE, Wilde M, Bilyeu TA, Burgess-Beusse BL, Finegold MJ, Darlington GJ** 1997 CCAAT/enhancer binding protein α regulates p21 protein and hepatocyte proliferation in newborn mice. Mol Cell Biol 17:7353–7361