Effect of a Gene Replacement on Mitochondrial Biogenesis in Fat Cells

Abstract

CCAAT/enhancer-binding proteins, C/EBPα and C/EBPβ, are required for fat cell differentiation and maturation. Previous studies showed that replacement of C/EBPα with C/EBPβ generating the ß/ß alleles in the mouse genome, prevents lipid accumulation in white adipose tissue (WAT). In this study, ß/ß mice lived longer and had higher energy expenditure than their control littermates due to increased WAT energy oxidation. The WAT of ß/ß mice was enriched with metabolically active, thermogenic mitochondria known for energy burning. The ß/ß allele exerted its effect through the elevated expression of the G protein stimulatory subunit (Gs) in WAT. Gs, when over-expressed in fat-laden fat cells, stimulated mitochondrial biogenesis similar to that seen in the WAT of ß/ß mice and effectively diminished the stored lipid pool.

Introduction

At its simplest level, obesity is a disorder of energy balance, where energy intake exceeds energy expenditure; as a consequence the excess energy is stored in the form of fat in adipocytes. The primary onset mechanisms are dietary and/or genetic (Kopelman 2000). Of those known genetic factors involved in determining adiposity in animals, many are regulatory and exert their effect directly on adipocyte differentiation and development (Flier 2004; Rosen et al. 2000). These factors include CCAAT/enhancer binding proteins (C/EBPs), SREBP and PPARγ, whose concerted action during adipogenesis leads to the development of fat-laden mature adipocytes (Rosen et al. 2000).

The C/EBP family consists of 5 members of which C/EBPα, C/EBPβ and C/EBPδ have a profound impact on fat cell differentiation (Yeh et al. 1995). During adipogenesis, C/EBP family functions in a transcriptional cascade in which the early and transiently expressed C/EBPβ and C/EBPδ activate transcription of PPARγ. PPARγ is then responsible for the expression of C/EBPα (Rosen et al. 1999; Wu et al. 1996). Subsequently, PPARγ and C/EBPα, together with SREBP, work synergistically to trans-activate expression of most or all of the genes encoding for factors, such as fatty acid synthase and adipocyte-specific fatty acid binding protein aP2, which characterize the fat cell phenotype (Speigelman et al. 1993).

In this study, we used previously manipulated gene knock-in mice, known as ß/ß mice, in which the C/EBPα gene has been replaced by the C/EBPβ gene (referred here as ß/ß allele) to study their function in tissues (Chen et al. 2000). ß/ß mice are lean, and despite markedly reduced fat storage in their fat cells, they do not develop hyperlipidemia or fatty liver, commonly found in the forced leanness that typically causes the liver to take up and store fatty acids when their circulating levels are elevated (Chen et al. 2000; Moitra et al. 1998; Shimomura et al. 1998). We monitored closely the physi-
ology and lifespan of the lean β/β mice and undertook mechanistic studies to understand the effect of β/β allele in energy metabolism. We found that the β/β allele caused an increase in mitochondrial biogenesis only in fat cells of white adipose tissues (WAT), and this WAT-specifically enhanced mitochondrial biogenesis was possibly elicited by the markedly elevated expression of G protein stimulatory subunit (Gs).

**β/β Mice Live Longer and Resist to Diet-Induced and Genetic Obesities**

To better understand the physiology of lean β/β mice, we monitored their lifespan, energy expenditure and responses to diet-induced stress. The average longevity of β/β mice (28.9±5.7 months; Fig. 1A) was 5.2 months longer than that of their heterozygous littermates (23.7±3.9 months; Fig. 1A). β/β mice consumed and produced significantly more O₂ and CO₂, respectively, than their heterozygous littermates at the ages examined, indicating that β/β mice expend more energy than their heterozygous littermates. To examine their response to dietary stress, β/β mice were fasted or fed a high fat (HF) diet. Despite losing more of their body weight than their heterozygous littermates during the 48 hr fasting period, they recovered it with a speed similar to that of their heterozygous littermates. This indicated that the lean state of β/β mice did not affect their ability to sustain and recover from fasting stress. When fed a high fat diet (30% fat), the heterozygous control mice gained 25% more weight than those fed the standard diet. By contrast, although β/β mice consumed more HF food (19% more) than their heterozygous littermates throughout the 6-week period, they did not gain more weight than those fed the standard diet, indicating that the β/β mice are protected from diet-induced weight gain.

We next introduced the β/β alleles into Lep<sup>+/+</sup> mice. Lep<sup>+/+</sup> mice are obese in part because of an excessive food intake (Naggett et al. 1995; Zhang et al. 1994). β/β x Lep<sup>+/+</sup> pups, carrying homozygous β/β alleles, appeared normal prior to weaning, but grew much slower than their Lep<sup>+/+</sup> littermates (Fig.1B). Weight gain in
B/Bl Lep<sup>−/−</sup> mice was markedly reduced. At 40 weeks of age, Lep<sup>−/−</sup> mice of both sexes were more than 150% heavier than their heterozygous littermates, while the male and female B/Bl Lep<sup>−/−</sup> mice were only 85% and 45% heavier, respectively. B/Bl Lep<sup>−/−</sup> mice had increased longevity (23.1±4.7 months) compared to their Lep<sup>−/−</sup> littermates (18±4 months; Fig.1A). Taken together, homozygous B/Bl alleles efficiently prevented marked weight gain associated with the loss of Lep gene function.

**β/β Mice Have a Higher Metabolism in Their Fat Tissue**

To assess the energy expenditure profile of β/β mice, we assayed various β/β mouse tissues for the expression of genes involved in mitochondrial oxidative respiration, such as cytochrome c oxidase (COX), and their enzymatic activity and found that the white adipose tissue (WAT, the main fat storage tissue) was the only tissue to possess elevated gene expressions and enzymatic activities for energy burning and thermogenesis (Fig. 2A). Structurally, the mitochondrial content of regular WAT adipocytes is low. However, electron microscopy showed that the mitochondrial volume was dramatically increased and full of straight or slightly wavy cristae that transverse the width of the mitochondria in the WAT adipocytes of β/β mice as compared to that of their heterozygous littermates (Fig. 2B) indicating a high metabolism in the WAT adipocytes of β/β mice.

**Gs Gene Expression is Elevated in the WAT of β/β Mice**

To understand the molecular mechanism by which β/β allele increase the metabolic activity in the WAT of β/β mice, we analyzed the WAT gene expression profile and found that the levels of mRNAs for the growth hormone receptor (GHR) and β3 adrenergic receptor (AR), were significantly reduced in WAT, while the mRNA levels for insulin receptor (IR) remained unaffected. This indicated that growth hormone and adrenergic factor signal transductions might be compromised in WAT of β/β mice. Interestingly, the expression of Gs, that couples with growth factor receptors such as β-AR (Neves et al. 2002) to mediate their activation, was markedly increased in WAT of β/β mice (Fig 2B). Also increased were the cellular levels of cAMP, an intracellular signal transducer for the activated Gs, which was
65% higher in the WAT of β/β mice (18.1 ± 2.3 pmol/mg protein) than that of β/+ mice (11.7 ± 1.4 pmol/mg protein).

**Gs Gene Expression Stimulate Mitochondria Biogenesis and Facilitate Fat Reduction in Fat Cells**

We next studied the direct effect of Gs on lipid-rich cells. Gs gene were delivered into lipid-rich 3T3-L1 cells via a recombinant adenoviral vector (Ad) carrying a GFP expression cassette, allowing infected cells to be identified (He et al. 1998). The infected lipid-rich 3T3-L1 cells were separated from non-infected cells in the same culture using flow cytometry and analyzed with their electron micrography, O2 consumption (an indicator of metabolic activity) and lipid accumulation. The Ad.Gs-infected cells showed higher O2 consumption than uninfected cells, while cells infected with Ad carrying only GFP (Ad.Track), PGC-1, GHR-S, mt-TOM20 (translocase of mitochondrial outer membrane, subunit 20) or a truncated form of Gs lacking the N-terminal 36 amino acid residues required for membrane targeting and direct contact with Gß dimer (Evanko et al. 2000; indicated as tr-Gs) did not exhibit any change in O2 consumption. This indicates that Gs gene has a stimulatory effect on cellular metabolic activity. When examined by electron microscopy, only the cells infected with Ad.Gs contained enlarged mitochondria full of cristae similar to those seen in the WAT of β/β mice, (Fig. 3). Furthermore, upon light microscopic analysis of cellular lipid accumulation, cells infected with Ad.Gs were found to lose their cellular lipid droplets by graduate shrinkage, whilst uninfected cells and those infected with Ad carrying other factors contained lipid droplets that were progressively enlarged and later merged together (Fig 4). Taken together, over-expression of Gs effectively increased mitochondrial volume and O2 consumption and subsequently reduced lipid accumulation in lipid-rich cells. This suggests that Gs might play an active role in programming the lipid-rich cells to be efficient in energy oxidation.

**Conclusion**

This study indicates that the β/β allele changes the metabolic state of WAT adipocytes from energy storage to energy dissipation, possibly via an increased expression of Gs. The increase in energy oxidation alone in fat cells appears to be able to reverse both genetic and dietary obesities. Regardless of the cause of obesity, all forms of obesity lead to an accumulation of massive quantities of fat in WAT. Thus, increasing the oxidative activity of WAT might be an effective treatment for obesity. Moreover, since over-expression alone of Gs effectively increased mitochondrial bio-
genesis and prevented fat accumulation in lipid-rich cells, Gs might be used to program the lipid-rich cells to be an efficient energy oxidizers.

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Reference


Figure 4. Gs gene reduce lipid accumulation in lipid-rich fat cells. Light micrograph of fat cells infected with adenoviral vectors expressing Gs proteins. Each micrograph shows both light (left) and dark (right, fluorescent) fields in parallel. Black and White arrowheads point to the same cell shown in light and dark fields, respectively. The numbers on the left of the micrograph indicate the number of days after adipogenic induction.

References


