Relationship between Human Adipose Tissue Agouti and Fatty Acid Synthase (FAS)[1,2]

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ABSTRACT The human homologue of the murine obesity gene, *agouti*, is expressed in adipose tissue. We have shown that recombinant agouti protein regulates adipocyte lipogenesis and lipolysis coordinately and promotes lipid storage via a Ca^{2+}-dependent mechanism in vitro, which may contribute to agouti-induced obesity. However, little is known about agouti's physiologic function in humans. We first studied the agouti content in human mature adipocytes vs. preadipocytes. The agouti content of human mature adipocytes was five times as abundant as in preadipocytes (19.18 ± 2.46 vs. 4.07 ± 0.51 pg/μg protein, $P < 0.005$), suggesting that agouti is up-regulated during adipocyte differentiation. We next studied the relationship of agouti mRNA and protein to fatty acid synthase (FAS) mRNA and activity in adipose tissue obtained from nonobese and mildly obese patients (body mass index range, 21–31 kg/m^2). Agouti protein was correlated with FAS activity ($r = 0.782$, $P < 0.005$). Similarly, human adipose tissue agouti mRNA level was also correlated with FAS mRNA level ($r = 0.846$, $P < 0.001$). These data suggest that agouti may be another adipocyte-produced factor that modulates adipocyte lipid metabolism via a paracrine/autocrine mechanism. J. Nutr. 130: 2478–2481, 2000.

KEY WORDS: • *agouti* • *fatty acid synthase* • *adipose tissue* • *adipocyte differentiation* • *obesity* • *humans*

The mouse *agouti* gene encodes a 131 amino acid secreted protein that normally regulates pigment production via a paracrine mechanism during its transient expression in neonatal skin (Galbraith 1964, Jackson 1991). However, ectopic expression of *agouti* in animal models such as A^y and A^{vy} or in transgenic mice overexpressing *agouti* under the control of β-actin promoter results in a yellow coat color, as well as obesity, hyperinsulinemia, insulin resistance, increased body length and susceptibility to neoplasia, which is collectively called "yellow obese" syndrome (Klebig et al. 1995, Yen et al. 1994).

Much attention has been directed toward central mechanisms of agouti's action. Recent evidence indicates that chronic antagonism of central melanocortin 4-receptor may be responsible for the hyperphagia and obesity in yellow agouti mutants (Fan et al. 1997, Huszar et al. 1997). However, the human homologue of *agouti* is expressed in both adipose tissue (Kwon et al. 1994) and pancreas (Xue et al.1999) in which it exerts important peripheral effects.

We showed previously that recombinant agouti protein exhibits potent lipogenic and antilipolytic effects in human adipocytes via a calcium-dependent mechanism (Jones et al. 1996, Kim et al. 1996, Xue et al. 1998), thereby promoting lipid storage in adipose tissue. Previous data from our laboratory also demonstrated that agouti protein stimulates calcium signaling and serves as a potent insulin secretagogue in both the pancreatic β-cell line and human pancreatic islets (Xue et al. 1999). Accordingly, we propose that this effect of agouti on insulin release, combined with its effect on adipocyte lipid metabolism, may represent a peripheral mechanism for agouti in the development of obesity. Consistent with this, although transgenic mice expressing *agouti* only in adipose tissue did not become obese, hyperinsulinemia in these mice produced by either daily insulin injection (Mynatt et al. 1997) or sucrose feeding (Zemel et al. 1999) did produce significant obesity in the transgenic mice, but not in their nontransgenic littermates. However, this hypothesis is based on in vitro studies of human adipocytes and human pancreatic islets. Little is known regarding its significance in vivo.

In this study, we investigated the relationship between agouti expression and adipocyte fatty acid synthase (FAS) expression and activity. We report here that human adipocyte agouti is up-regulated during human preadipocyte differentiation. In addition, there is a strong correlation between human adipose tissue agouti protein content and FAS activity as well as between human adipose tissue agouti mRNA and FAS mRNA level.

MATERIALS AND METHODS

Cell culture. Human subcutaneous preadipocytes were supplied by Zen-Bio (Research Triangle Park, NC). To differentiate into mature adipocytes, preadipocytes were plated at a density of 30,000 cells/cm^2 with preadipocyte medium [Dulbecco's modified Eagle me-

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dium (DMEM)/Ham's F-10 nutrient broth (Ham's F-10), 1:1 (v/v) containing 10% fetal calf serum, 15 mmol/L HEPES and antibiotics] and allowed to grow to confluence in 1–2 d. Cells were then induced to differentiate with differentiation medium [DMEM/Ham's F-10, 1:1 (v/v) containing 10% fetal bovine serum, 15 mmol/L HEPES, 33 μmol/L biotin, 17 μmol/L pantothenate, 100 nmol/L insulin, 1 μmol/L dexamethasone, 0.25 mmol/L isobutyl methylxanthine, 1 μmol/L BRL49653 and antibiotics]. After 3 d of differentiation, cells were maintained in adipocyte medium [DMEM/Ham's F-10, 1:1 (v/v) containing 3% fetal bovine serum, 15 mmol/L HEPES, 33 μmol/L biotin, 17 μmol/L pantothenate, 100 nmol/L insulin, 1 μmol/L dexamethasone and antibiotics] until fully differentiated.

Human adipose tissue. Human subcutaneous adipose tissue was obtained during liposuction from nonobese and mildly obese patients, with body mass indices ranging from 21 to 31 kg/m^2. This protocol was approved by the Institutional Review Board for Human Subjects and the Committee for Research Participation of the University of Tennessee.

Agouti protein measurement. Human preadipocytes and mature adipocytes were rinsed once with Hank's balanced salt solution (Life Technologies, Grand Island, NY) and homogenized in sucrose buffer (250 mmol/L sucrose, 1 mmol/L dithiothreitol, 1 mmol/L EDTA and 100 μmol/L phenylmethylsulfonyl fluoride). Human adipose tissue was homogenized in sucrose buffer. Fat-free infranatant was obtained by centrifugation at 18,500 × *g* for 60 min using a Sorval RMC 14 microcentrifuge (Sorval, Newtown, CT). Agouti protein was measured using whole-cell homogenates from pre- and mature adipocyte preparations and the fat-free infranatant from human adipose tissue via an agouti RIA kit (Phoenix, Mountain View, CA), similar to the approach used recently for the assessment of tissue leptin content (Claycombe et al. 2000).

Fatty acid synthase (FAS) activity determination. FAS activity was determined spectrophotometrically using fat-free infranatant from human adipose tissue by measuring the oxidation rate of NADPH as described previously (Moustaid et al. 1988).

Protein measurement. Total protein was determined by a modified Bradford method using Coomassie blue dye (Pierce, Rockford, IL) (Bradford 1976).

Northern blot analysis. Total RNA from human adipose tissue was isolated using $CsCl_2$ density centrifugation. RNA from different fat samples were electrophoresed on the same gel (1%) and transferred to nylon membranes (New England Nuclear, Boston, MA). Membranes were hybridized with ^{32}P-labeled human FAS, agouti and β-actin (Clontech, Palo Alto, CA) cDNA probe at 42°C overnight using Ultra Hyb solution (Ambion, Austin, TX). Membranes were washed with 2X saline-sodium citrate (SSC) at 42°C for 15–30 min, 0.1X SSC at 42°C for 15–30 min and then 0.1X SSC at 60°C for 30–60 min. Finally, membranes were exposed to X-ray film (New England Nuclear) at −80°C. Autoradiograms were analyzed by densitometric scanning.

Statistics. All data are expressed as means ± SEM Data were analyzed using the procedures of the SPSS (Chicago, IL). Different agouti protein content in pre- and mature adipocyte was compared by independent sample *t* test. The relationships between adipose tissue agouti expression and FAS expression and agouti protein and FAS activity were analyzed by linear regression. Pearson correlation coefficients were calculated to determine the relationship between variables. A *P*-value < 0.05 was considered significant.

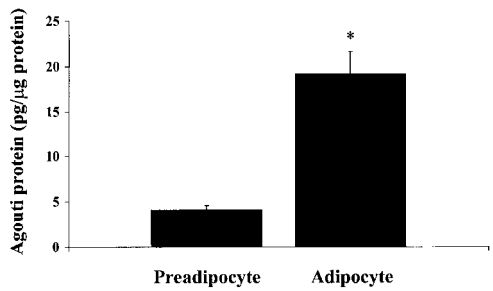

FIGURE 1 Effect of human adipocyte differentiation on cellular agouti protein content, *$P < 0.005$. Values are means ± SEM, $n = 6$.

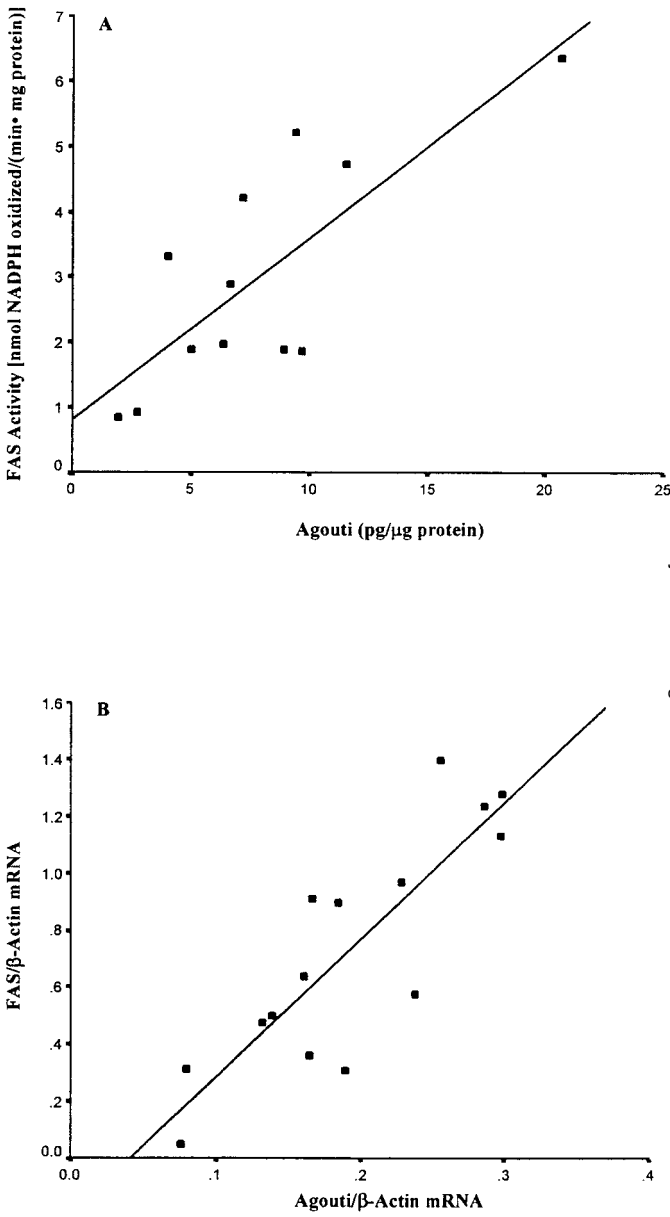

FIGURE 2 Correlation between agouti protein and fatty acid synthase (FAS) activity (*panel A*) and between agouti mRNA and FAS mRNA level (*panel B*) in human adipose tissue. *Panel A*: $r = 0.782$, $P < 0.005$. *Panel B*: $r = 0.846$, $P < 0.001$.

RESULTS

We first studied the effect of human adipocyte differentiation on adipocyte agouti levels. As shown in **Figure 1**, agouti protein level was four times more abundant in human mature adipocytes than in preadipocyte (19.18 ± 2.46 vs. 4.07 ± 0.51 pg/μg protein, $P < 0.005$), suggesting that agouti is up-regulated during human adipocyte differentiation.

We next examined the relationship between human adipose tissue agouti expression and FAS expression and activity. Human adipose tissue obtained from liposuction patients contained quantities of agouti (**Fig. 2**A) comparable to those found in adipocytes obtained by differentiating cultured human stromal vascular preadipocytes (Fig. 1). Human adipose

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tissue agouti protein content varied >10-fold among different individuals (Fig. 2A). Over this range, adipose tissue agouti content was correlated with FAS activity (Fig. 2A, $r = 0.782$, $P < 0.005$).

Similarly, agouti mRNA level in human adipose tissue varied over fourfold among different individuals, and was correlated with FAS mRNA levels (Fig. 2B, $r = 0.846$, $P < 0.002$).

DISCUSSION

The human homologue of *agouti*, unlike its murine counterpart, is expressed in adipose tissue (Kwon et al. 1994), indicating a possible physiologic role for agouti in human adipose tissue. We showed previously that the FAS mRNA level is significantly higher in adipose tissue from obese A^{vy} mice than in lean (*a/a*) controls (Jones et al. 1996). In addition, recombinant agouti protein stimulated both the expression and activity of adipocyte FAS in vitro via a Ca^{2+}-dependent mechanism (Jones et al. 1996). Further, the FAS promoter contains an agouti response element that also responds to KCl, a membrane depolarization agent (Claycombe et al. 1997). We also demonstrated that agouti protein inhibited both basal- and agonist-stimulated lipolysis in human adipose tissue via a Ca^{2+}-dependent mechanism (Xue et al. 1998). These in vitro data suggest a coordinated regulation of adipocyte lipid metabolism by agouti, which may in turn promote lipid storage in adipose tissue.

Numerous reports have demonstrated that adipose tissue synthesizes various proteins and hormones, participating in energy regulation through a network of endocrine, paracrine and autocrine signals [reviewed in Fried and Russel (1998) and Mohamed-Ali et al. (1998)]. Leptin, for example, is a satiety factor produced by adipocyte and regulates body weight through its effects on food intake in hypothalamus. In addition, adipose tissue has been also shown to produce factors that act in an endocrine and paracrine/autocrine manner to modulate its own metabolism, preadipocyte proliferation and differentiation, and multiple systemic effects. These factors include the cytokines, interleukin-6 and tumor necrosis factor-α, acylation-stimulating protein, angiotensin II, prostaglandins, sex steroids and plasminogen activator inhibitor-1 [reviewed in Fried et al. (1998), Hwang et al. (1997) and Mohamed-Ali et al. 1998)].

Fatty acid synthase is the key enzyme in de novo lipogenesis. Both human liver and adipose tissue exhibit substantially lower FAS activity than that found in rats (Weiss et al. 1986). Nonetheless, significant de novo lipogenesis is well documented in human adipocytes. Human adipocytes contain substantial levels of FAS activity which is sensitive to both nutritional and hormonal modulation (Moustaid et al. 1996). Studies in humans fed a high carbohydrate diet demonstrated that total body fat synthesis significantly exceeded hepatic de novo lipogenesis, suggesting that adipose tissue may be the major site for fat synthesis (Aarsland et al. 1997). Further, another study showed that adipose tissue accounts for up to 40% of whole-body lipogenesis under this condition (Chascione et al. 1987). The effect of carbohydrate intake on adipose tissue lipogenesis is qualitatively similar in humans and in rats. Quantitatively, rats have higher rates of lipogenesis, which may be explained in part by the higher metabolic rate in rats than in humans (Chascione et al. 1987). Moreover, FAS levels are elevated in obese animals (Guichard et al. 1992). Thus, induction of adipocyte lipogenesis may contribute to obesity.

In this study, we demonstrated that human adipocyte agouti is up-regulated during differentiation. In addition, we showed a strong correlation between agouti expression and FAS expression and activity in human adipose tissue. It is possible that agouti and FAS are regulated in a similar manner. FAS is regulated primarily by hormonal and nutritional factors at the transcription level. It is increased by feeding, thyroid hormone and insulin, and decreased by starvation, cAMP and polyunsaturated fatty acids [reviewed in Sul and Wang (1998)]. However, it is not known at present which factor(s) may modulate adipocyte agouti expression and how it is regulated. Further, because agouti is a paracrine/autocrine factor that does not enter the general circulation (Wolff 1963) and because it has been shown to regulate FAS expression and activity in vitro (Jones et al. 1996), it is possible that agouti may be another adipocyte-synthesized factor, which may contribute in part to the regulation of adipocyte lipid metabolism.

In summary, we have found that human adipocyte agouti is up-regulated during adipocyte differentiation. In addition, there is a strong correlation between human adipose tissue agouti protein content and FAS activity as well as between human adipose tissue agouti mRNA and FAS mRNA level. Consistent with this, we have shown that agouti regulates adipocyte lipid metabolism in vitro (Jones et al. 1996, Xue et al. 1998). These data suggest that agouti may be an additional adipocyte factor, which modulates adipocyte lipid metabolism via a paracrine/autocrine mechanism.

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