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Characterization of the Fasting-induced Adipose Factor FIAF, a Novel Peroxisome Proliferator-activated Receptor Target Gene*

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Fasting is associated with significant changes in nutrient metabolism, many of which are governed by transcription factors that regulate the expression of rate-limiting enzymes. One factor that plays an important role in the metabolic response to fasting is the peroxisome proliferator-activated receptor α (PPAR α). To gain more insight into the role of PPAR α during fasting, and into the regulation of metabolism during fasting in general, a search for unknown PPAR α target genes was performed. Using subtractive hybridization (SABRE) comparing liver mRNA from wild-type and PPAR α null mice, we isolated a novel PPAR α target gene, encoding the secreted protein FIAF (for fasting induced adipose factor), that belongs to the family of fibrinogen/angiopoietin-like proteins. FIAF is predominantly expressed in adipose tissue and is strongly up-regulated by fasting in white adipose tissue and liver. Moreover, FIAF mRNA is decreased in white adipose tissue of PPAR γ +/- mice. FIAF protein can be detected in various tissues and in blood plasma, suggesting that FIAF has an endocrine function. Its plasma abundance is increased by fasting and decreased by chronic high fat feeding. The data suggest that FIAF represents a novel endocrine signal involved in the regulation of metabolism, especially under fasting conditions.

In many developed and developing countries, the prevalence of diabetes, particularly type II diabetes, is increasing at an alarming rate. Despite intensive research over the past decades, the knowledge about the metabolic derangements precipitating to and accompanying type II diabetes remains fragmentary. One factor that has limited progress of diabetes research has been a lack of clear understanding of the regulation of nutrient metabolism under normal, non-diabetic conditions. Indeed, much still needs to be learned about the genetics of metabolism during various physiological states, such as fasting.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF278699.

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Fasting can be described as a state when food intake has been arrested for a significant amount of time. The absence of energy entering the body evokes a complex physiological response aimed at maintaining whole body homeostasis. A critical event in the fasting response is the liberation of fatty acids from the adipose tissue and their preferential use as an energy substrate in tissues such as skeletal muscle and liver. The metabolic adaptations accompanying fasting are governed by numerous endocrine and cellular factors. Fasting results in pronounced changes in the plasma concentrations of important metabolic hormones such as insulin, glucocorticoids, leptin, and glucagon. In addition, fasting causes altered expression levels of important transcription factors, such as sterol response element-binding protein (1), c-Myc (2), and peroxisome proliferator-activated receptor α (PPAR α)¹ (3), directing specific changes in the expression of metabolic enzymes. We and others (3, 4) have recently shown the important role of PPAR α in the fasting response. By stimulating the oxidation of fatty acids in liver, this transcription factor assures an efficient output of ketone bodies and glucose by the liver.

Besides PPAR α , two other PPARs (β and γ) are currently known (5). All belong to the superfamily of nuclear hormone receptors, which encompasses a large collection of transcription factors that regulate transcription in response to small lipophilic compounds such as retinoic acid, vitamin D, thyroid hormone, and fatty acids (6). Like other nuclear hormone receptor, PPARs bind to the promoter of target genes and stimulate transcription after binding of a specific ligand. In the case of PPAR α , a variety of fatty acids and fatty acid derivatives have been shown to bind and activate PPAR α . This includes long-chain unsaturated fatty acids such as linoleic acid; branched, conjugated, and oxidized fatty acids such as phytanic acid and conjugated linoleic acid; and eicosanoids such as 8(S)-hydroxyeicosatetraenoic acid and leukotriene B₄ (5).

Functionally, the PPAR α and PPAR γ isoforms have been relatively well characterized. For PPAR α , it has been shown, both *in vitro* and *in vivo*, that this receptor plays an important role in the oxidation of fatty acids in the liver by activating the expression of many genes involved in this metabolic pathway (7, 8). PPAR α also mediates the effect of peroxisome proliferators on hepatic cell proliferation, as demonstrated by the observation that mice that lack PPAR α are refractory to the effects of peroxisome proliferators (9, 10). Whereas PPAR α

¹ The abbreviations used are: PPAR, peroxisome proliferator-activated receptor; FIAF, fasting-induced adipose factor; SABRE, selective amplification via biotin and restriction-mediated enrichment; GFP, green fluorescent protein; bp, base pair(s); kb, kilobase(s); ORF, open reading frame; WAT, white adipose tissue; BAT, brown adipose tissue; HFARP, hepatic fibrinogen/angiopoietin-related protein; PAGE, polyacrylamide gel electrophoresis.

functions in lipid catabolism, the role of PPAR γ seems to be geared toward anabolic pathways, particularly in adipose tissue. Activation of PPAR γ in preadipocytes is sufficient to direct the cell toward the adipocyte differentiation program (11), leading to dramatic build-up of lipid inside the cell. Other tissues where PPAR γ may play a role include the colon and monocytes/macrophages where PPAR γ may play a role in cytokine production (12).

Studies with PPAR α null mice have shown that PPAR α becomes especially important during fasting (3, 4). When fasted, these mice suffer from a defect in fatty acid oxidation and ketogenesis, resulting in elevated plasma free fatty acid levels, hypoketonemia, hypothermia, and hypoglycemia. To gain more insight into the function of PPAR α and to increase our understanding of the genetic regulation of metabolism during fasting, an unbiased subtractive hybridization assay was performed to search for unknown PPAR α target genes. Here we describe the isolation and characterization of a novel PPAR α target gene, FIAF, for fasting-induced adipose factor, implicated in the metabolic response to fasting.

EXPERIMENTAL PROCEDURES

Animals—Mice were housed in a temperature-controlled room (23 °C) on a 10-h dark, 14-h light cycle. Pure-bred wild-type or PPAR α -null mice on a sv129 background were used (7). Animal experiments were approved by the animal authorization commission of the canton of Vaud (Switzerland). Fasted animals were deprived of food for 24 h, starting at the beginning of the light cycle. Fed animals were sacrificed at the end of the dark cycle. WY14643 was administered by dissolving it in the drinking water (0.1%) for 3 weeks. The high fat diet (39% fat energy) was high in saturated fat (coconut oil-based, ICN Research Diets) and followed for 15 weeks.

Generation of the PPAR γ +/- mice will be described elsewhere. Briefly, the A/B domain and the 5'-end of the first zinc finger were deleted, resulting in removal of the translation start site. Both wild-type and mutant mRNA can be detected in PPAR γ +/- mice.

SABRE—SABRE (selective amplification via biotin and restriction-mediated enrichment) employs selective streptavidin-biotin affinity and restriction enzyme site reconstitution to enrich for cDNA species more abundant in one population than in another (13). One male sv129 wild-type mouse and one male PPAR α null mouse of about 8 months of age were sacrificed in the middle of the light cycle. Experimental details on preparation of cDNA and further analysis by SABRE will be described elsewhere.

RNA Preparation and Northern Blots—Total RNA was prepared from frozen livers, white adipose tissue, and brown adipose tissue using the RNeasy midikit (Qiagen) or from other tissues using Trizol reagent (Life Technologies, Inc.). 15–30 μ g of total RNA was loaded per lane. Electrophoresis, blotting, and hybridization were done according to standard protocols. The cDNA of the ribosomal protein L27 was used as a control probe. Labeling was carried out using a High Prime kit (Roche Molecular Biochemicals).

RACE—The full-length sequence of the cDNA of mouse FIAF was obtained by 5'- and 3'-RACE reactions using a Marathon cDNA amplification kit (CLONTECH). The sequences of the primers used were: 5'-RACE, GGTCCCCACGGAGGTCATGGTCTTGG; 3'-RACE, GGGT-GAGGACACAGCCTACAGCCTGC. Mouse liver cDNA was used as the template.

Transfections—The open reading frame of mouse FIAF was subcloned into the green fluorescence protein (GFP) fusion vector pEGFP-N2 (CLONTECH), which contains a strong cytomegalovirus promoter, to create the mFIAF-GFP fusion construct (GFP is located toward the C terminus). To obtain mFIAF not fused to GFP, the termination codon of mFIAF was left intact. HEK293 cells were cultured in Dulbecco's modified Eagle's medium with 5% fetal calf serum. Cells were transfected with the vectors indicated above by calcium-phosphate precipitation. Immediately after transfection, medium was replaced by Dulbecco's modified Eagle's medium without calf serum. 24 h after transfection, the medium was harvested, centrifuged to remove remaining cells, and stored at -20 °C. Protein was precipitated by adding 8 volumes of cold acetone and leaving the medium at -20 °C for several hours. Protein was pelleted by centrifugation and, after washing, redissolved in SDS-PAGE loading buffer. SDS-PAGE was further performed according to standard procedures.

Western Blot—The polyclonal antibody used was directed against the epitope CQGPKGKDAPFKDSE in the N-terminal region of FIAF. The peptide affinity-purified antibody was generated in rabbit and ordered via Eurogentec's customized antibody production service. Western blotting was carried out using an ECL system (Amersham Pharmacia Biotech) according to the manufacturer's instructions. The primary antibody was used at a dilution of 1:1000, the secondary antibody (anti-rabbit IgG, Sigma) was used at a dilution of 1:8000. All incubations were performed in TBS, pH 7.5, with 0.1% Tween 20 and 5% dry milk, except for the final washings when the dry milk was omitted.

RNase Protection Assay—A BamHI/EcoRI fragment was excised off a partial L27 cDNA and subcloned into pBluescript KS (Stratagene). The resulting vector, pBS-L27, was linearized by EcoRI and transcribed by T7 RNA polymerase yielding a 200-base riboprobe and a protected fragment of 150 bp. A 300-bp fragment containing the 5'-end of FIAF was subcloned into pBluescript KS. A 356-base antisense riboprobe was synthesized by T7 RNA polymerase, resulting in a 300-bp protected fragment. For FIAF probe a ratio of 1:1 of [α^{32} -P]UTP to cold UTP was used, whereas a 1:20 ratio was used for the L27 probe. Incorporation and specific activity of each probe was determined after purification via RNeasy Clean-Up (Qiagen).

Direct lysate ribonuclease protection assay was carried out as described by the manufacturer (Ambion) with some modifications (14). Briefly, tissues were homogenized with a Polytron homogenizer in lysis/denaturation solution (2 mg/ml) and further clarified by centrifugation via a Qiashreder (Qiagen). 20 μ l of lysate was hybridized to 10 ng of specific FIAF probe (1×10^9 cpm/mg) and 10 ng of L27 probe (1×10^8 cpm/mg). RNase digestion (10 units/ml RNase A; 400 units/ml RNase T1) was carried out for all probes at 37 °C for 20 min. The products of ribonuclease protection assay were resolved in a 6% electrolyte-gradient denaturing polyacrylamide gel.

Collection of Blood Plasma—Blood was drawn by retro-orbital puncture. Blood was collected into heparinized tubes, kept on ice, and spun for 10 min to collect plasma. Plasma was kept at -70 °C.

RESULTS

To search for unknown genes regulated by PPAR α , we compared liver mRNA from wild-type and PPAR α null mice by performing the subtractive hybridization assay SABRE described above (13). One of the cDNA fragments isolated corresponded to an unknown gene (further referred to as FIAF for reasons explained below), whose differential expression between wild-type and PPAR α null mice was evident by Northern blot (Fig. 1A). To obtain the full-length mouse cDNA of FIAF, 5'- and 3'-RACE were carried out using internal primers of the 600-bp cDNA fragment (Fig. 1B). Primer extension revealed that the 5'-RACE fragment stopped about 25 bp short of the transcriptional start site. The genomic sequence could be directly extracted from the GenBank[®] data base (accession number AF110520.1), because the mouse FIAF gene happens to be located in the mouse major histocompatibility complex II locus on chromosome 17, in a region that has been completely sequenced. With the genomic sequence information, the core promoter containing a putative TATA box could be located. The mouse FIAF gene contains seven exons and six introns, spanning about 7 kb (Fig. 1C). From the full-length cDNA an ORF of 1233 bp could be derived, giving rise to a protein of 410 amino acids and a predicted molecular mass of around 46 kDa (Fig. 1B). Further analysis of the primary sequence revealed similarity with a family of proteins that contain a so-called fibrinogen-like domain. In addition, a coiled-coil domain that typically precedes the fibrinogen-like domain was present. The highest similarity was found with a group of angiopoietins and with ficolin-A. Angiopoietins have been implicated in endothelial developmental processes, whereas ficolin seems to bind sugar residues on bacterial surfaces. However, the identity at the amino acid level did not exceed 24%, suggesting that the function of FIAF is likely to be different from the angiopoietins and from ficolin. In analogy with other fibrinogen-like proteins, a putative signal sequence was found at the N terminus of FIAF possibly directing secretion of the protein. To investigate whether the protein was indeed secreted, the ORF was sub-

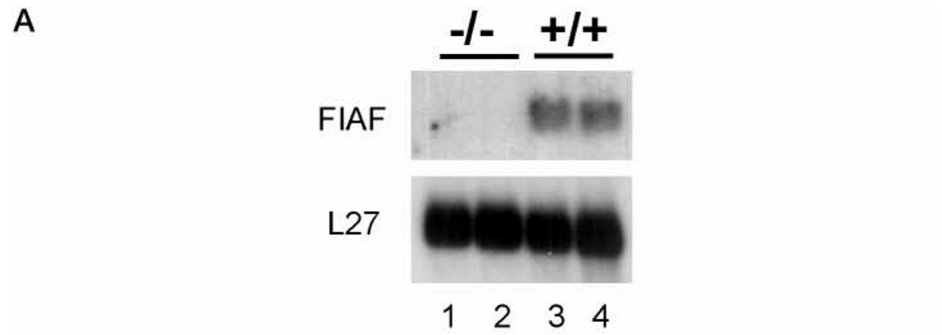
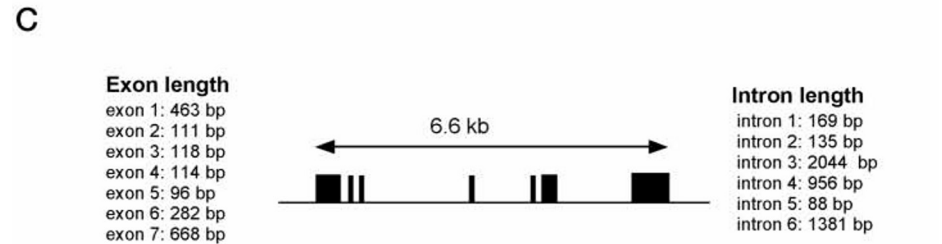
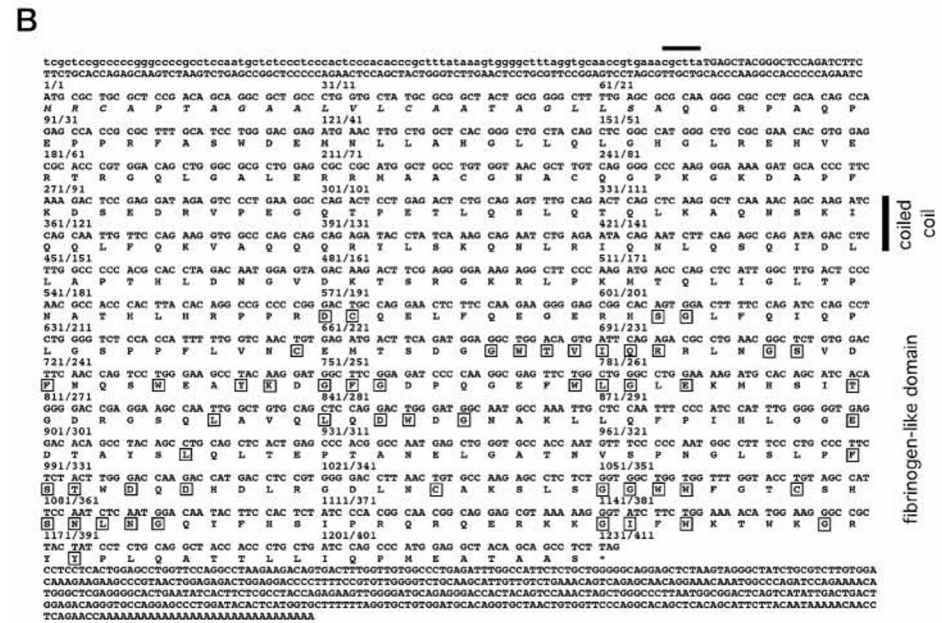


FIG. 1. A, FIAF cannot be detected in livers of PPAR α null mice. Northern blot was performed with liver RNA from wild-type and PPAR α null mice killed in the middle of the light cycle. B, cDNA and amino acid sequence of mouse FIAF. Full-length cDNA is given in uppercase letters. The promoter sequence is given in lowercase letters. The numbering starts at the translation start site. The approximately 25 base pairs missing at the 5'-end of the original 5'-RACE fragment, as indicated by primer extension, were taken from the genomic sequence and have been added to the cDNA sequence. The approximate transcriptional start site is indicated by a bar. The signal sequence, as determined by computer analysis using the signalP program, is shown in *italics*. Amino acid residues identical with at least five of the following six most related mouse proteins are boxed: angiotensin-1, angiotensin-2, angiotensin-3, ficolin-A, angiotensin-related protein 2, and angiotensin-related protein 3. C, genomic organization of the mouse FIAF gene. The gene contains seven exons and six introns, spanning 6.6 kb. The start codon is present in the first exon.



cloned behind a cytomegalovirus promoter and the vector was transfected into HEK293 cells. The ORF was also linked to green fluorescent protein to create a GFP fusion protein. A polyclonal antibody directed against a non-conserved peptide in the N-terminal region was synthesized. As shown in Fig. 2, FIAF could be detected in the culture medium by Western blot, both as the GFP fusion protein (*lane 2*), and as the native protein (*lane 3*), indicating that FIAF is indeed secreted.

Although FIAF was cloned from a liver cDNA library, it is probably expressed elsewhere as well. To examine the expression profile of FIAF, Northern blot was performed using RNA from a variety of tissues. Interestingly, FIAF was very highly expressed in white adipose tissue and to a somewhat lesser extent in brown adipose tissue (Fig. 3). Other tissues such as lung, kidney, and liver also expressed FIAF but at much lower levels. No signal could be detected in heart, skeletal muscle, and brain. Taken together, the data show that FIAF encodes a secreted, angiotensin/fibrinogen-like protein, primarily produced by the adipose tissue.

Because FIAF was less expressed in liver of PPAR α null mice than in wild-type mice, it is possible that FIAF is a target gene

of PPAR α and of synthetic PPAR α ligands such as fibrates. To study the effect of PPAR α on FIAF expression, mice were treated with the potent PPAR α ligand WY14643. In wild-type mice but not in PPAR α null mice, WY14643 increased expression of FIAF, providing further evidence that FIAF is a PPAR α target gene in liver (Fig. 4A). To study the factors influencing the expression of FIAF and thereby provide some clues about the possible function of this protein, the effect of fasting was examined. Fasting was chosen because it is accompanied by an increase in PPAR α expression (3). Moreover, the effects of PPAR α deletion become more manifest during fasting (3, 4). Interestingly, fasting caused a dramatic increase in FIAF expression in liver, but the PPAR α dependence was completely lost (Fig. 4B). A marked increase in FIAF expression was observed in white adipose tissue (WAT) as well. For this reason the protein was named FIAF, for fasting induced adipose factor. Remarkably, in WAT FIAF expression was higher in PPAR α null mice than in wild-type mice.

WAT contains very little PPAR α (15). In contrast, the PPAR γ isotype is very abundant in WAT (15). To examine whether expression of FIAF in adipose tissue is under control of PPAR γ ,

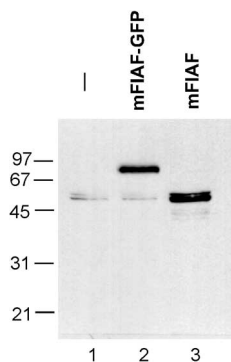


FIG. 2. **mFIAF is a secreted protein.** HEK293 cells were transfected with an empty vector (*lane 1*), a vector expressing a fusion between mFIAF and green fluorescent protein (*lane 2*), or an expression vector for mFIAF alone (*lane 3*). 24 h after transfection the culture medium was collected and prepared for SDS-PAGE (170 μ l of medium/lane). Western blotting was performed with a peptide-purified antibody directed against a specific epitope in the N-terminal region of mFIAF.

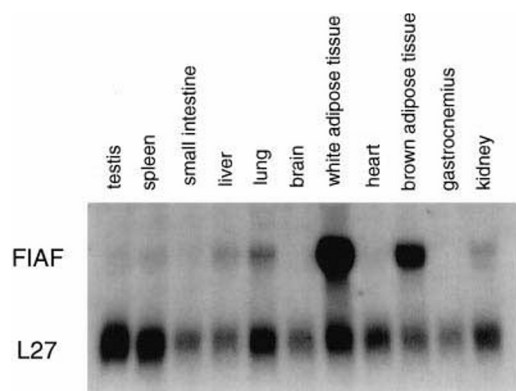


FIG. 3. **mFIAF is predominantly expressed in adipose tissue.** Northern blotting was performed with RNA derived from a variety of mouse tissues. Full-length mFIAF cDNA was used as a probe.

FIAF mRNA was determined in WAT of heterozygous PPAR γ mutant mice. Heterozygous PPAR γ mutant mice were chosen, because homozygous null mice are embryonically lethal (16, 17). As shown in Fig. 5, mFIAF mRNA expression was down-regulated in PPAR γ +/- mice.

Because FIAF is a secreted protein, it was of interest to investigate whether it may be circulating in the blood plasma. Interestingly, using the purified antibody against FIAF in Western blotting, a band could be detected in plasma at exactly the same molecular weight as FIAF protein produced in cell culture, suggesting that it corresponds to FIAF (Fig. 6A). This band was also observed in liver, kidney, WAT, and brown adipose tissue (BAT), tissues where FIAF is produced (Fig. 6, A and B). In kidney, BAT, and WAT, another band was observed with a slightly higher molecular weight than the original band. Most likely, this slower moving band represents FIAF immediately after synthesis, with the signal sequence still attached. It is also possible that the two bands represent different glycosylated forms of the protein, which have been previously shown to occur for the angiopoietins. To corroborate that the band observed in plasma truly represented FIAF protein, the effect of fasting was examined. Fasting caused an approximate 2-fold increase in the intensity of the putative FIAF band, in accordance with the mRNA expression data showing an increased FIAF expression during fasting (Fig. 6C). Interestingly, prolonged feeding of a high fat diet, which was accompanied by an average 2.2-fold increase in epididymal fat mass, decreased the intensity of the putative FIAF band by about 2-fold (Fig. 6D). Modulation of FIAF abundance in plasma was, however, not

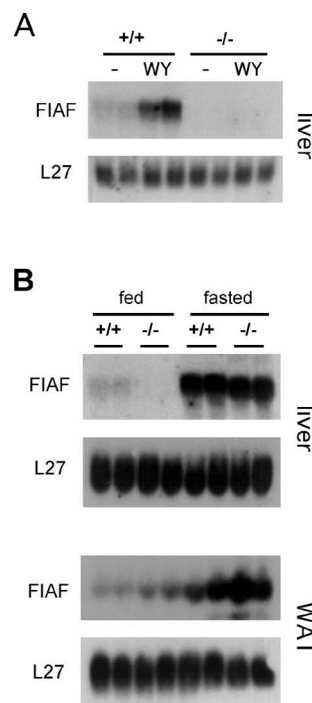


FIG. 4. **Expression of mFIAF is regulated by PPAR α and by fasting.** A, Northern blot of liver RNA from wild-type and PPAR α null mice treated with WY14643. B, Northern blot of liver and white adipose tissue (WAT) RNA from fed and fasted wild-type and PPAR α null mice.

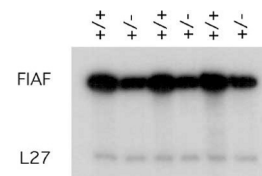


FIG. 5. **FIAF mRNA expression is decreased in WAT of heterozygous PPAR γ mutant mice.** 8- to 12-week-old PPAR γ +/+ and +/- mice were sacrificed at the end of the dark cycle (fed state). RNase protection was performed directly on WAT tissue extracts using a probe corresponding to the first 300 bp of the full-length cDNA.

associated with altered expression of mRNA levels in liver (Fig. 7A) or WAT (Fig. 7B).

DISCUSSION

Besides being important for the storage and subsequent release of fatty acids, research over the past decade has shown that white adipose tissue also has an important endocrine function. Factors such as plasminogen activator inhibitor I, tumor necrosis factor α , and leptin are produced by the adipose tissue to exert effects elsewhere in the body. Here we describe the cloning of a novel fibrinogen/angiopoietin-like factor, primarily secreted from the adipose tissue, whose production is elevated during fasting and whose mRNA expression is stimulated by PPAR α in liver and by PPAR γ in WAT.

In recent years, a wealth of data has accumulated about leptin, the product of the mouse *ob* gene. Initially viewed as a sensor of the fat stores that acts on the hypothalamus to inhibit food intake, leptin is now realized to have a much broader role in energy homeostasis (18). This is exemplified by the observation that the expression of leptin in WAT is acutely reduced by fasting (19, 20), suggesting that leptin is also involved in short term metabolic regulation. In contrast to leptin, expression of FIAF is up-regulated during fasting. Furthermore, the abundance of FIAF in plasma is decreased with high fat feeding and increased WAT mass, an effect directly opposite to that observed with leptin (18). Finally, leptin is a negative target of

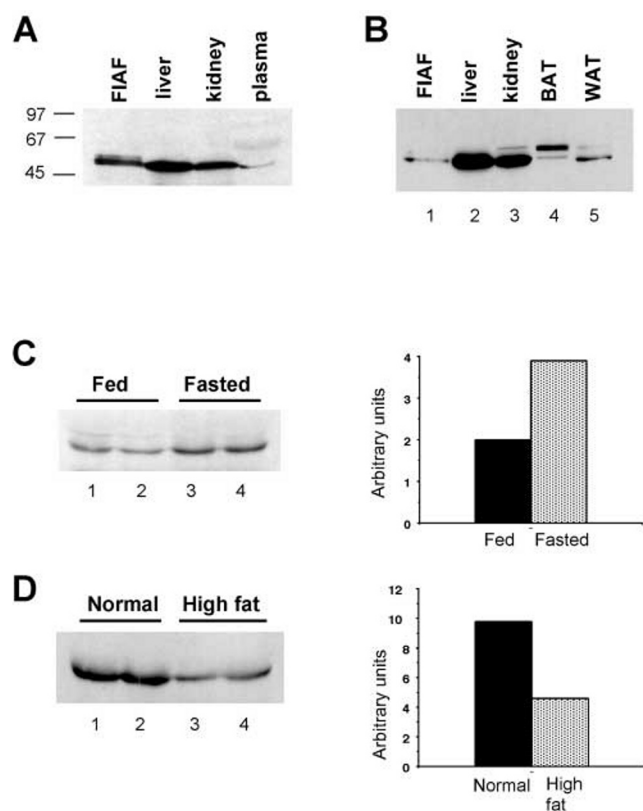


FIG. 6. FIAF protein is present in plasma and regulated by fasting and high fat feeding. *A*, a band detected in plasma by Western blotting moves exactly at the same molecular weight as FIAF produced in cell culture and detected in liver and kidney. Amounts of material loaded are as follows; *lane 1*, 170 μ l of culture medium of HEK293 cells transfected with mFIAF; *lane 2*, 300 μ g of liver tissue; *lane 3*, 300 μ g of kidney tissue; *lane 4*, 1 μ l of mouse blood plasma. *B*, FIAF protein can be detected in a variety of tissues. 300 μ g of tissue was loaded for liver, kidney, and brown adipose tissue (BAT). For WAT, the amount of tissue loaded could not be calculated, but the amount of protein was adjusted to similar levels as in *lanes 2–4* by comparing Ponceau staining of the blot after transfer. *C*, FIAF abundance in plasma is regulated by fasting. 1 μ l of blood plasma of fed or fasted wild-type mice was loaded per lane. Relative quantifications of the bands are shown in the accompanying *bar graph*. *D*, FIAF abundance in plasma is regulated by high fat feeding. 1 μ l of blood plasma of mice fed a normal or high fat diet for 15 weeks was loaded per lane.

PPAR γ and is overexpressed in WAT of PPAR γ +/- mice (16, 21, 22), whereas FIAF mRNA is down-regulated in these mice. Although it remains speculative to suggest that FIAF and leptin have antagonistic functions, the opposite regulation between leptin and FIAF expression/production during various nutritional states and by PPAR γ is nevertheless very interesting.

Our data clearly show that the expression of FIAF is strongly increased during fasting in both liver and WAT. This, together with the observation that expression of FIAF is increased by PPAR α , whose critical involvement in the adaptive response to fasting has been well established (3, 4), suggests that FIAF is mainly important under fasting conditions. Interestingly, PPAR α deletion had no effect on FIAF expression in the liver of fasted mice, a phenomenon that was previously observed for other PPAR α target genes such as short chain acyl-CoA dehydrogenase and carnitine palmitoyl acyl-transferase I, which are genes whose expression is strongly stimulated by fasting as well (3). Most likely, the loss of PPAR α dependence can be ascribed to other signaling pathways activated during fasting, which override the effect of PPAR α . FIAF expression was found to be somewhat higher in WAT of PPAR α null mice than wild-type mice (mainly evident in the fed state). This could

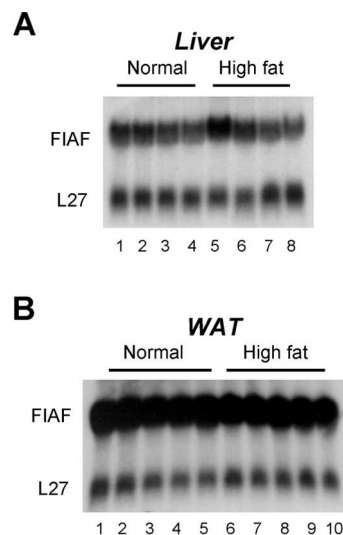


FIG. 7. mRNA expression of FIAF in liver and white adipose tissue is not affected by high fat feeding. *A*, Northern blot of liver RNA of mice fed a normal or high fat diet for 15 weeks. *B*, Northern blot of WAT RNA of mice fed a normal or high fat diet for 15 weeks.

either reflect a negative regulation of FIAF expression by PPAR α in WAT (either directly or via other transcription factors) or a consequence of the metabolic derangements present in PPAR α null mice. Increased expression levels of genes implicated in metabolism in WAT of PPAR α null mice have been observed previously.² Future research will have to establish more precisely what role FIAF plays in the metabolic response to fasting.

The closest family members of FIAF, the angiopoietins, are secreted proteins that acts as ligands for receptor-like tyrosine kinases such as TIE2 (23, 24). Similar to the angiopoietins, it is likely that FIAF exerts its effect distant from its site of production, probably by binding to some kind of receptor. The detection of FIAF in plasma suggests that FIAF's mode of action is endocrine, rather than autocrine or paracrine, although this remains to be proven. At the present time, no information is available regarding the molecular target of FIAF or about its target tissue(s).

It was observed that the abundance of FIAF in plasma is decreased on a chronic high fat diet. Remarkably, this was not accompanied by changes in mRNA expression in two major FIAF-producing tissues, liver and WAT. Besides being possibly caused by post-transcriptional effects, it is also conceivable that a high fat diet lowers plasma FIAF abundance by increasing its rate of breakdown. In the absence of any information on the identity and location of the putative FIAF receptor, this hypothesis would be very difficult to test.

It is remarkable that, despite much higher mRNA expression levels in WAT, FIAF protein seems to be more highly present in liver and kidney. One possibility that could explain this apparent discrepancy is that FIAF protein is stored in the latter tissues, whereas it is immediately secreted after synthesis in WAT. Alternatively, it is conceivable that the concentrated presence of FIAF protein in liver and kidney reflects uptake rather than production. Again, to test this hypothesis, more information about the target tissues of FIAF is needed.

While this manuscript was in preparation, Kim *et al.* (25) reported on a hepatic fibrinogen/angiopoietin-related protein (HFARP) that is identical to our FIAF. As was found for FIAF, HFARP was secreted by COS-7 cells and was detected in circulating blood. The highest expression of HFARP was found in

² S. Kersten and W. Wahli, unpublished data.

the liver, but WAT and BAT, where we found much higher expression levels, were omitted. According to these authors, HFARP acts as an apoptosis survival factor for vascular endothelial cells. It is not easy to reconcile this function with the predominant expression of FIAF/HFARP in adipose tissue and its marked up-regulation by fasting. Given the large divergence in sequence between FIAF and angiopoietins, these proteins are likely to have quite different functions.

In conclusion, we have isolated a novel gene that is a PPAR α target gene in liver, and probably a PPAR γ target gene in WAT, that encodes the secreted protein FIAF. Its high expression in adipose tissue and its up-regulation by fasting suggest an involvement of FIAF in the metabolic response to fasting. Moreover, its plasma levels are reduced by chronic high fat feeding. Future studies are needed to establish more precisely the role of FIAF in energy homeostasis.

REFERENCES

- Horton, J. D., Bashmakov, Y., Shimomura, I., and Shimano, H. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 5987–5992
- Corcos, D., Vaulont, S., Denis, N., Lyonnet, S., Simon, M. P., Kitzis, A., Kahn, A., and Kruh, J. (1987) *Oncogene Res.* **2**, 193–199
- Kersten, S., Seydoux, J., Peters, J. M., Gonzalez, F. J., Desvergne, B., and Wahli, W. (1999) *J. Clin. Invest.* **103**, 1489–1498
- Leone, T. C., Weinheimer, C. J., and Kelly, D. P. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96**, 7473–7478
- Desvergne, B., and Wahli, W. (1999) *Endocr. Rev.* **20**, 649–688
- Gronemeyer, H., and Laudet, V. (1995) *Protein Profile* **2**, 1173–1308
- Lee, S. S., Pineau, T., Drago, J., Lee, E. J., Owens, J. W., Kroetz, D. L., Fernandez-Salguero, P. M., Westphal, H., and Gonzalez, F. J. (1995) *Mol. Cell. Biol.* **15**, 3012–3022
- Peters, J. M., Aoyama, T., Cattley, R. C., Nobumitsu, U., Hashimoto, T., and Gonzalez, F. J. (1998) *Carcinogenesis* **19**, 1989–1994
- Peters, J. M., Hennuyer, N., Staels, B., Fruchart, J.-C., Fievet, C., Gonzalez, F. J., and Auwerx, J. (1997) *J. Biol. Chem.* **272**, 27307–27312
- Aoyama, T., Peters, J. M., Iritani, N., Nakajima, T., Furihata, K., Hashimoto, T., and Gonzalez, F. J. (1998) *J. Biol. Chem.* **273**, 5678–5684
- Wu, Z., Puigserver, P., and Spiegelman, B. M. (1999) *Curr. Opin. Cell Biol.* **11**, 689–694
- Ricote, M., Huang, J. T., Welch, J. S., and Glass, C. K. (1999) *J. Leukocyte. Biol.* **66**, 733–739
- Lavery, D. J., Lopez-Molina, L., Fleury-Olela, F., and Schibler, U. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 6831–6836
- Haines, D. S., and Gillespie, D. H. (1992) *BioTechniques* **12**, 736–741
- Braissant, O., Foufelle, F., Scotto, C., Dauca, M., and Wahli, W. (1996) *Endocrinology* **137**, 354–366
- Kubota, N., Terauchi, Y., Miki, H., Tamemoto, H., Yamauchi, T., Komeda, K., Satoh, S., Nakano, R., Ishii, C., Sugiyama, T., Eto, K., Tsubamoto, Y., Okuno, A., Murakami, K., Sekihara, H., Hasegawa, G., Naito, M., Toyoshima, Y., Tanaka, S., Shiota, K., Kitamura, T., Fujita, T., Ezaki, O., Aizawa, S., Nagai, R., Tobe, K., Kimura, S., and Kadowaki, T., et al. (1999) *Mol. Cell* **4**, 597–609
- Barak, Y., Nelson, M. C., Ong, E. S., Jones, Y. Z., Ruiz-Lozano, P., Chien, K. R., Koder, A., and Evans, R. M. (1999) *Mol. Cell* **4**, 585–595
- Friedman, J. M., and Halaas, J. L. (1998) *Nature* **395**, 763–770
- Trayhurn, P., Thomas, M. E., Duncan, J. S., and Rayner, D. V. (1995) *FEBS Lett.* **368**, 488–490
- Vidal-Puig, A., Jimenez-Linan, M., Lowell, B. B., Hamann, A., Hu, E., Spiegelman, B., Flier, J. S., and Moller, D. E. (1996) *J. Clin. Invest.* **97**, 2553–2561
- Hollenberg, A. N., Susulic, V. S., Madura, J. P., Zhang, B., Moller, D. E., Tontonoz, P., Sarraf, P., Spiegelman, B. M., and Lowell, B. B. (1997) *J. Biol. Chem.* **272**, 5283–5290
- De Vos, P., Lefebvre, A. M., Miller, S. G., Guerre-Millo, M., Wong, K., Saladin, R., Hamann, L. G., Staels, B., Briggs, M. R., and Auwerx, J. (1996) *J. Clin. Invest.* **98**, 1004–1009
- Suri, C., Jones, P. F., Patan, S., Bartunkova, S., Maisonpierre, P. C., Davis, S., Sato, T. N., and Yancopoulos, G. D. (1996) *Cell* **87**, 1171–1180
- Davis, S., Aldrich, T. H., Jones, P. F., Acheson, A., Compton, D. L., Jain, V., Ryan, T. E., Bruno, J., Radziejewski, C., Maisonpierre, P. C., and Yancopoulos, G. D. (1996) *Cell* **87**, 1161–1169
- Kim, I., Kim, H.-G., Kim, H., Kim, H.-H., Park, S. K., Uhm, C.-S., Lee, Z. L., and Koh, G. Y. (2000) *Biochem. J.* **346**, 603–610