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Previews

FGF21: A Missing Link in the Biology of Fasting

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A sufficient energy supply is essential for life; consequently, multiple mechanisms have evolved to ensure both energy availability and conservation during fasting and starvation. Two reports in this issue of *Cell Metabolism* (Badman et al., 2007; Inagaki et al., 2007) demonstrate that FGF21, a circulating protein produced in the liver in response to the PPARα transcription factor, is a "missing link" in the biology of fasting, inducing adipose tissue lipolysis, liver ketogenesis, and metabolic adaptation to the fasting state.

Biology of Fasting

The adaptation from the fed to fasted (total lack of food intake, usually acute) or starved (chronic undernutrition) state has been a subject of fascination, and investigation, for centuries (Cahill, 2006; Keys et al., 1950; see also Tucker, 2006). In the fed state, glucose fulfills the body's acute, immediate energy needs. The body senses a drop in glucose concentration at sites such as the pancreatic islets, brain, and portal vein. It responds by reducing insulin secretion from islet β cells and by increasing glucagon secretion from islet α cells. Another response is sympathetic adrenal stimulation causing increased epinephrine levels; this arm is of secondary importance but has a larger role in patients with type 2 diabetes (Cryer et al., 2003).

During fasting, liver glycogen, a glucose-storage polymer, is initially mobilized to replenish blood glucose (glycogenolysis). Major changes in metabolism occur as the glycogen supply dwindles. Stored adipose tissue triglycerides are released into the circulation as glycerol and fatty acids. The glycerol is converted by the liver into glucose (gluconeogenesis). The fatty acids are directly oxidized as an energy source by some tissues (liver and muscle); the liver also metabolizes the fatty acids to β-hydroxybutyrate and acetoacetate ("ketone bodies"). Ketone bodies are released into the circulation for use by tissues, notably the brain, which cannot use fatty acids. The liver also uses ketones for gluconeogenesis. When fasting is prolonged, muscle protein breakdown occurs, sending alanine to the liver as another substrate for gluconeogenesis (Cahill, 2006).

The major controllers of energy homeostasis are insulin, glucagon, and the sympathetic nervous system, but other regulator hormones play a role too. Leptin is secreted by adipose tissue in proportion to adipose triglyceride content (the determinant of how long the body can survive starvation); this information on triglyceride levels controls chronic energy homeostasis and related events, including, for example, the ability to reproduce. Other physiological signals of energy status include the gut hormones that communicate nutritional status (examples include ghrelin, cholecystokinin, pancreatic polypeptide, glucagon-like peptide-1, polypeptide YY, gastrin-releasing peptide, and neuromedin U). None of these gut hormones, however, has the dramatic, nonredundant importance of insulin, glucagon, or leptin, as evidenced by the deficiency phenotypes of the respective knockout mice. Hypothetical roles for as yet undiscovered hormones might include hormones secreted by the liver reporting its glycogen or triglyceride content, or by muscle broadcasting that its glycogen stores are low or that its lactate production is high.

Over the past few years, it has become clear that there is another source of "hormonal" information on fuel homeostasis. There are now a number of examples of the metabolic fuels themselves serving as hormones (i.e., as ligands without undergoing further metabolism). Metabolic substrate G protein-coupled receptors include GPR109A for β-hydroxybutyrate, GPR91 for succinate, GPR99 for α-ketoglutarate, and GPR41 and GPR43 for short-chain fatty acids. Intracellular transcription factors that appear to be fuel sensors are HNF4 and PPARs for fatty acids (among others; see Lazar and Willson, 2007).

FGF21

FGF21 burst into the picture when Kharitonenkov et al. (2005) showed that it improved glucose, insulin, and triglyceride levels in diabetic mice and that transgenic overexpression resulted in a lean, insulin-sensitive phenotype. A follow-up study of 6 weeks' treatment of diabetic rhesus monkeys significantly expanded these results: FGF21 reduced glucose, insulin, and glucagon levels; improved lipid profiles; and slightly reduced body weight (Kharitonenkov et al., 2007). Of significant interest for identifying possible pharmaceutical targets, no signals of hypoglycemia or cell proliferation were detected.

The two papers in this issue (Badman et al., 2007; Inagaki et al., 2007) establish FGF21 as a hormone important in the intermediate time period (hours) in the body's adaptation to fasting. The papers complement each other well, with Inagaki et al. concentrating on FGF21 excess, showing that it is sufficient, while Badman et al. focus on FGF21 deficiency (liver knockdown), showing that it is necessary, for adaptation to fasting (Figure 1).

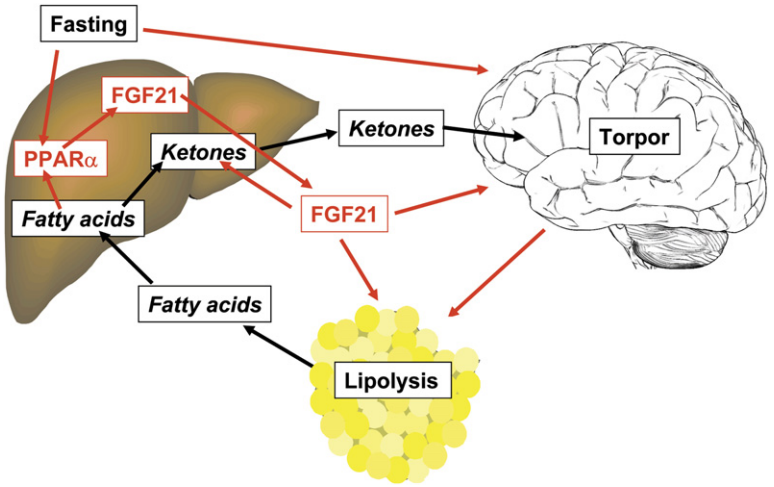

Figure 1. Biology of FGF21 in Liver, Brain, and Adipose Tissue
Fuels are shown in black italics, and regulators are shown in red. FGF21 actions on liver are depicted as endocrine but could also be paracrine. Fasting is detected by the brain, leading to lipolysis and contributing to other adaptations such as torpor. Fatty acids are released from adipose tissue, taken up by the liver, and either oxidized or converted to ketones. Ketones released by the liver are used as fuel by the brain. Activation of PPARα, presumably via activation by fatty acids, increases transcription of FGF21. FGF21 contributes to ketogenesis in liver, lipolysis in adipose, and adaptation such as torpor by the brain.

Both groups place FGF21 downstream of the transcription factor PPARα (the target of the fibrate class of triglyceride-lowering drugs), which has been identified as a key regulator of the adaptation to fasting (Lefebvre et al., 2006). They report that fasting or PPARα agonist treatment increased liver FGF21 mRNA levels about 25-fold. *PPARα*$^{-/-}$ mice showed reduced basal FGF21 mRNA levels with no response to PPARα agonist and only a partial increase in FGF21 mRNA upon fasting. Inagaki et al. (2007) demonstrate PPAR binding elements in the *FGF21* promoter, suggesting that this is a direct effect, possibly mediated by fatty acid binding to PPARα. They show that in the fed state, FGF21 increases adipose tissue lipolysis and hepatic ketogenesis, increasing circulating β-hydroxybutyrate and reducing glucose, insulin, and cholesterol levels (confirming the findings of Kharitonenkov et al., 2005). The effects of FGF21 in the fed state mimic the adaptation to the fasted state seen in control mice. *PPARα*$^{-/-}$ mice are known to have an abnormal response to fasting, including defective fatty acid oxidation and ketogenesis (Kersten et al., 1999), defects that were partially remedied by FGF21 dosing. FGF21 also caused adipose lipolysis, providing fatty acids to the liver for ketogenesis, and induced torpor, a state characterized by physical inactivity and a low body temperature. Small mammals normally require a high metabolic rate in order to maintain body temperature but use torpor as an adaptive strategy to conserve energy when food is scarce.

Badman et al. (2007) identify FGF21 as a liver mRNA induced in mice subjected to fasting, fed a ketogenic diet, or treated with a PPARα agonist. Circulating FGF21 protein levels increased (about 2-fold) with fasting and returned to fed levels by 4 hr of refeeding but were only modestly reduced in the *PPARα*$^{-/-}$ mice. Interestingly, the elevated FGF21 mRNA levels of the ketogenic diet were not reduced in the *PPARα*$^{-/-}$ mice, indicating that induction of FGF21 can also be independent of PPARα. Adenovirus knockdown of liver FGF21 expression in mice on a ketogenic diet caused fatty liver, lipemia, and reduced serum ketones, with reductions in liver RNAs for fatty acid oxidation and ketogenesis genes. The combined observations that FGF21 regulates adipose (lipolysis), liver (fatty acid oxidation and ketogenesis), and brain (torpor) establish it as a major endocrine regulator of the response to fasting.

What Are the Unanswered Questions?

As with any new hormone, there is much to learn about FGF21's biology. For example: What role does it have in other ketogenic states, such as the newborn period, lactation, and pregnancy? Are some effects of FGF21 autocrine or paracrine, rather than endocrine? Does FGF21 reach the brain directly, or are its actions there indirect? A fascinating observation is that some seizure disorders that are unresponsive to other therapies are effectively treated by a ketogenic diet (Freeman et al., 2007). There is no clear mechanistic understanding for this success. Presumably FGF21 is induced in these patients. Might it also have a role in their treatment?

FGF21 is but one member of a 22-member family. However, three members, FGF21, FGF19 (Fgf15 in the mouse), and FGF23, constitute a distinct subfamily and share a number of attributes atypical for FGFs. The three appear to be classical endocrine hormones, being made in distinct sites (FGF21 in liver, FGF19/Fgf15 in intestine, and FGF23 in osteoblast) and acting distantly (FGF19/Fgf15 represses bile acid synthesis and inhibits gallbladder emptying; FGF23 regulates phosphaturia and vitamin D metabolism). These FGFs bind heparin relatively poorly, presumably facilitating their endocrine rather than paracrine actions and their need for Klotho/β-Klotho receptor accessory proteins (Goetz et al., 2007).

What receptor (or receptors) is FGF21 acting through? There are four FGF receptor genes with tyrosine kinase activity, with multiple splice isoforms that affect specificity and thus receptor function. Additionally, receptor activity can be dependent on receptor-associated proteins. A recent paper shows that β-Klotho, a transmembrane protein that associates with FGF receptors 1c and 4, is required for FGF21 signaling in 3T3-L1 adipocytes (Ogawa et al., 2007). This is an exciting development that should help answer selectivity and specificity questions.

Is FGF21 a good drug candidate for diabetes, dyslipidemia, and/or obesity? The favorable diabetes and lipid phenotypes are encouraging. The rhesus studies (Kharitonenkov et al., 2007) suggest that these effects are not rodent specific (for example, due to the higher rodent liver PPARα levels or the importance of active brown adipose tissue). On the safety front, the lack of detected hypoglycemia or cell proliferation is important. Thus, this mechanism seems worthy of further investigation.

The observations reported in this issue by Badman et al. (2007) and Inagaki et al. (2007) identify FGF21 as an important endocrine hormone helping control adaptation to the fasted state. This provides a previously missing link, downstream of PPARα, by which the liver communicates with the rest of the body in regulating the biology of energy homeostasis.

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REFERENCES

Badman, M.K., Pissios, P., Kennedy, A.R., Koukos, G., Flier, J.S., and Maratos-Flier, E. (2007). Cell Metab. *5*, this issue, 426–437.

Cahill, G.F., Jr. (2006). Annu. Rev. Nutr. *26*, 1–22.

Cryer, P.E., Davis, S.N., and Shamoon, H. (2003). Diabetes Care *26*, 1902–1912.

Freeman, J.M., Kossoff, E.H., and Hartman, A.L. (2007). Pediatrics *119*, 535–543.

Goetz, R., Beenken, A., Ibrahimi, O.A., Kalinina, J., Olsen, S.K., Eliseenkova, A.V., Xu, C., Neubert, T.A., Zhang, F., Linhardt, R.J., et al. (2007). Mol. Cell. Biol. *27*, 3417–3428.

Inagaki, T., Dutchak, P., Zhao, G., Ding, X., Gautron, L., Parameswara, V., Li, Y., Goetz, R., Mohammadi, M., Esser, V., et al. (2007). Cell Metab. *5*, this issue, 415–425.

Kersten, S., Seydoux, J., Peters, J.M., Gonzalez, F.J., Desvergne, B., and Wahli, W. (1999). J. Clin. Invest. *103*, 1489–1498.

Keys, A., Brozek, J., Henschel, A., Mickelsen, O., and Taylor, H.L. (1950). The Biology of Human Starvation (Minneapolis: University of Minnesota Press).

Kharitonenkov, A., Shiyanova, T.L., Koester, A., Ford, A.M., Micanovic, R., Galbreath, E.J., Sandusky, G.E., Hammond, L.J., Moyers, J.S., Owens, R.A., et al. (2005). J. Clin. Invest. *115*, 1627–1635.

Kharitonenkov, A., Wroblewski, V.J., Koester, A., Chen, Y.F., Clutinger, C.K., Tigno, X.T., Hansen, B.C., Shanafelt, A.B., and Etgen, G.J. (2007). Endocrinology *148*, 774–781.

Lazar, M.A., and Willson, T.M. (2007). Cell Metab. *5*, 159–161.

Lefebvre, P., Chinetti, G., Fruchart, J.C., and Staels, B. (2006). J. Clin. Invest. *116*, 571–580.

Ogawa, Y., Kurosu, H., Yamamoto, M., Nandi, A., Rosenblatt, K.P., Goetz, R., Eliseenkova, A.V., Mohammadi, M., and Kuro, O.M. (2007). Proc. Natl. Acad. Sci. USA *104*, 7432–7437.

Tucker, T. (2006). The Great Starvation Experiment: The Heroic Men Who Starved so That Millions Could Live (New York: Simon & Schuster Free Press).

Live Longer through PHAsting

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Dietary restriction provides considerable health benefits and may even increase life span in humans. Panowski et al. (2007) have now identified PHA-4/FoxA as an essential and specific component of DR-induced life-span extension in *C. elegans*.

The notion that life span is subject to the action of genes has become widely accepted. Despite the claims of popular anti-aging pills and creams, dietary restriction (DR) or calorie restriction is the only general nongenetic intervention that has been shown to improve health and increase life span in various model systems. DR differs from starvation and is usually defined as about 30% lower food intake than that of ad libitum feeding. In addition to DR, life span can be extended by reduced insulin/IGF-1 signaling (IIS) and reduced mitochondrial electron transport chain activity (ECT) (Figure 1) (reviewed in Wolff and Dillin, 2006).

A recent study by Panowski et al. (2007) now identifies the *C. elegans pha-4* gene as an essential and unique mediator of DR-induced life-span extension. The Dillin lab previously identified suppressor of MEK1 (SMK-1) as a regulator of DAF-16, a forkhead transcription factor homologous to mammalian FoxO that mediates IIS-induced longevity (Wolff et al., 2006). However, the role of DAF-16 in DR has remained enigmatic thus far, and surprisingly, it was observed that SMK-1, but not DAF-16, affected DR-induced life span. Because DR uncouples SMK-1 from DAF-16, the authors hypothesized that SMK-1 could regulate another forkhead transcription factor. By systematic screening of all 15 *C. elegans* forkheads, they identified PHA-4, the homolog of mammalian FoxA (HNF-3). Two independent DR models were used to demonstrate PHA-4 involvement. The classical genetic model for DR uses the mutant