AMP-activated protein kinase and coordination of hepatic fatty acid metabolism of starved/carbohydrate-refed rats

Murwarid M. Assifi,1 Gabriela Suchankova,1 Scarlet Constant,1 Marc Prentki,2 Assish K. Saha,1 and Neil B. Ruderman1

1Diabetes Unit, Section of Endocrinology and Departments of Medicine, Physiology and Biochemistry, Boston University Medical Center, Boston, Massachusetts; and 2Molecular Nutrition Unit, Departments of Nutrition and Biochemistry and the Montreal Diabetes Research Center, University of Montreal, Montreal, Quebec, Canada

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Assifi, Murwarid M., Gabriela Suchankova, Scarlet Constant, Marc Prentki, Assish K. Saha, and Neil B. Ruderman. AMP-activated protein kinase and coordination of hepatic fatty acid metabolism of starved/carbohydrate-refed rats. Am J Physiol Endocrinol Metab 289: E794–E800, 2005. First published June 14, 2005; doi:10.1152/ajpendo.00144.2005.—Acute starvation and refeeding are coordinated by AMPK. AMPK belongs to a family of highly conserved serine kinases that are regulated by nutritional and metabolic stresses that increase or decrease cellular levels of AMP and ATP and perhaps other molecules (16, 19). When activated, AMPK protects the cell against ATP depletion by stimulating processes such as fatty acid oxidation that promote ATP generation and inhibiting others that use ATP but are not acutely necessary for survival (16, 22). In keeping with this role, AMPK has been shown to affect the activity of a number of enzymes of lipid metabolism whose activity is altered by starvation and refeeding. For instance, activation of AMPK in liver has been shown to do the following: 1) to phosphorylate and inactivate acetyl-CoA carboxylase (ACC), leading to a decrease in the concentration of malonyl-CoA, an allosteric inhibitor of carnitine palmitoyltransferase I (CPT I) (7, 15, 36); 2) to suppress the transcriptional regulator sterol regulatory element-binding protein-1c (SREBP-1c), an effect that decreases the expression of ACC, fatty acid synthase (FAS) (61), and the first committed enzyme in the pathway of glycerolipid synthesis, sn-glycerol-3-phosphate acyltransferase (GPAT) (8, 35); and 3) to increase the activity (51) and expression (46) of CPT I. In addition, some, although not all, studies (14) have suggested that AMPK regulates the activity of malonyl-CoA decarboxylase (MCD), a major determinant of malonyl-CoA turnover in many tissues (42, 52), and that it does so both by phosphorylating and activating the enzyme (42) and by regulating its expression (10, 45). It has also been suggested that AMPK could regulate GPAT by altering its phosphorylation (35).

In the present study, we examined the possibility that AMPK coordinates the transition of hepatic lipid metabolism from catabolism to anabolism in the starved rat when it is refed. In addition, we assessed whether changes in the activity of ACC, as well as acetyl-CoA carboxylase (ACC), and other enzymes that govern fatty acid and glycerolipid synthesis relate temporally to alterations in the activities of the fuel-sensing enzyme AMP-activated protein kinase (AMPK). Rats starved for 48 h and refed a carbohydrate chow diet for 1, 3, 12, and 24 h were studied. Refeeding caused a 40% decrease in the activity of the α1-isofor of AMPK within 1 h, with additional decreases in AMPKα1 activity and a decrease in AMPKα2 occurring between 1 and 24 h. At 1 h, the decrease in AMPK activity was associated with an eightfold increase in the activity of the α1-isofor of ACC and a 30% decrease in the activity of MCD, two enzymes thought to be regulated by AMPK. Also, the concentration of malonyl-CoA was increased by 50%. Between 1 and 3 h of refeeding, additional increases in the activity of ACC and decreases in MCD were observed, as was a further twofold increase in malonyl-CoA. Increases in the activity (60%) and abundance (12-fold) of fatty acid synthase occurred predominantly between 3 and 24 h and increases in the activity of mitochondrial sn-glycerol-3-phosphate acyltransferase (GPAT) and acyl-CoA:diacylglycerol acyltransferase (DGAT) at 12 and 24 h. The results strongly suggest that early changes in the activity of MCD, as well as ACC, contribute to the increase in hepatic malonyl-CoA in the starved-refed rat. They also suggest that the changes in these enzymes, and later occurring increases in enzymes regulating fatty acid and glycerolipid synthesis, could be coordinated by AMPK.

The transition from the fasted to the fed state is associated with nutritional and hormonal changes that lead to increased hepatic glycerolipid and fatty acid synthesis and decreased fatty acid oxidation and ketogenesis (41). Many of the enzymes that mediate these events have been identified, as have the hormonal factors and molecular mechanism(s) by which their activity is altered (2, 8, 16, 17, 28, 45, 53). We examine here the possibility that the changes in activity of these enzymes during refeeding are coordinated by AMPK. AMPK belongs to a family of highly conserved serine kinases that are regulated by nutritional and metabolic stresses that increase or decrease cellular levels of AMP and ATP and perhaps other molecules (16, 19). When activated, AMPK protects the cell against ATP depletion by stimulating processes such as fatty acid oxidation that promote ATP generation and inhibiting others that use ATP but are not acutely necessary for survival (16, 22). In keeping with this role, AMPK has been shown to affect the activity of a number of enzymes of lipid metabolism whose activity is altered by starvation and refeeding. For instance, activation of AMPK in liver has been shown to do the following: 1) to phosphorylate and inactivate acetyl-CoA carboxylase (ACC), leading to a decrease in the concentration of malonyl-CoA, an allosteric inhibitor of carnitine palmitoyltransferase I (CPT I) (7, 15, 36); 2) to suppress the transcriptional regulator sterol regulatory element-binding protein-1c (SREBP-1c), an effect that decreases the expression of ACC, fatty acid synthase (FAS) (61); and the first committed enzyme in the pathway of glycerolipid synthesis, sn-glycerol-3-phosphate acyltransferase (GPAT) (8, 35); and 3) to increase the activity (51) and expression (46) of CPT I. In addition, some, although not all, studies (14) have suggested that AMPK regulates the activity of malonyl-CoA decarboxylase (MCD), a major determinant of malonyl-CoA turnover in many tissues (42, 52), and that it does so both by phosphorylating and activating the enzyme (42) and by regulating its expression (10, 45). It has also been suggested that AMPK could regulate GPAT by altering its phosphorylation (35).

In the present study, we examined the possibility that AMPK coordinates the transition of hepatic lipid metabolism from catabolism to anabolism in the starved rat when it is refed. In addition, we assessed whether changes in the activity of MCD, as well as ACC, could account for the increase in hepatic malonyl-CoA in the starved-refed rat. Toward these ends, the activity of the α1- and α2-isofor of AMPK was determined in liver of 48-h-starved rats before and after 1, 3, 12, and 24 h of refeeding a chow diet. In addition, the activity and, where possible, the abundance of key enzymes involved in regulating fatty acid metabolism, such as ACC1, MCD, FAS, GPAT, and DGAT were determined. The results indicate that the activity

* These authors contributed equally to this paper.

Address for reprint requests and other correspondence: N. B. Ruderman, Diabetes and Metabolism Unit, Boston Univ. Medical Center, 650 Albany St., EBRC-825, Boston, MA 02118 (e-mail: nrude@bumc.bu.edu).

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of AMPK is significantly diminished after 1 h of refeeding and remains low for at least an additional 23 h. They also reveal that these changes in AMPK antedate or are associated with changes in the activity of all of the above-mentioned enzymes. Finally, they demonstrate that a change in both MCD and ACC activity accompany the early increase in hepatic malonyl-CoA content during refeeding.

MATERIALS AND METHODS

Experimental animals. Male Sprague-Dawley rats (155–160 g; Harlan Sprague Dawley, Indianapolis, IN) were housed in individual cages in a room on a 12:12-h light-dark cycle and fed a rat chow diet containing 20% protein, 15% fat, and 65% carbohydrate (Harlan-Teklad, Madison, WI) ad libitum for 6 days. Rats were then divided into groups that were either starved for 48 h or starved and then refed for 1, 3, 12, or 24 h. After this, they were anesthetized with pentobarbital sodium (4 mg/100 g body wt ip), and portions of the liver were excised and frozen in liquid nitrogen for subsequent analyses. The activities of the α1- and α2-isoforms of AMPK and the α-isoform of ACC (ACC1) were determined as previously described (20, 36). MCD activity was measured fluorometrically after (NH4)2SO4 purification of the 14,000-g supernatant of a hepatic homogenate, as described by Park et al. (36). Protein concentration was determined by the method of Bradford with bovine serum albumin as the standard (6).

Western blot analysis. Liver supernatants containing 50 µg of protein, prepared as for activity assays, were electrophoresed and transferred to polyvinylidene difluoride membranes (Bio-Rad). After the membranes were blocked with 5% nonfat dry milk in TBST (25 mM Tris, 135 mM NaCl, 2.5 mM KCl, 0.05% Tween-20) overnight at 4°C, they were incubated with antibody to ACC (total; Upstate Biotechnology, Waltham, MA), MCD (NH2-terminal) (42), or FAS (BD Transduction Laboratories, San Jose, CA). Bands were visualized by enhanced chemiluminescence and were quantitated by laser densitometry.

GPAT, DGAT, and FAS activity assays. GPAT in liver homogenate (600-g supernatant) was assayed with 300 µM [3H]glycerol-3-phosphate and 80 µM palmitoyl-CoA in the presence or absence of 1 mM N-ethylmaleimide to inhibit the microsomal isoform of the enzyme as described by Muio et al. (35) and used by us previously (36). Results are presented as the incorporation of [3H]glycerol-3-phosphate into DGAT was assayed in 600-g liver homogenate in the presence of 1,2-diacyl-sn-glycerol dispersed in 0.1% Tween-20 and [14C]palmitoyl-CoA as described in Ref. 44. FAS activity was determined as described by Linn (29). The oxidation of NADPH was followed at 340 nm in a reaction mixture containing 90 mM phosphate buffer, 0.18 mM EDTA, 0.10 mM acetyl-CoA, 0.20 mM NADPH, and 0.20 mM malonyl-CoA. The reaction was initiated by the addition of malonyl-CoA following the addition of 600-g liver supernatant (29). Of the enzymes studied, only GPAT was assayed by 10.2 ± 0.3 on April 10, 2017 http://ajpendo.physiology.org/ Downloaded from

RESULTS

AMPK activity. We examined the effects of 48 h of starvation and 1–24 h of refeeding a standard high-carbohydrate chow diet on AMPK activity in liver. AMPK was found to be regulated acutely, with the α1-isofom being most responsive (Fig. 1A). Thus a 40% decrease in AMPKα1 activity was observed at 1 h with modest additional decreases occurring up to 12 h. A significant decrease in the activity of AMPKα2 was not observed after 1 h; however, thereafter, decreases in its activity paralleled those of AMPKα1, and by 24 h they were significantly lower than prefeeding values. AMPKα1 and AMPKα2 activities were reduced by 60 and 35%, respectively, compared with prefeeding values.

Malonyl-CoA, ACC, MCD, and FAS. As reported previously (7), hepatic malonyl-CoA levels increase rapidly after refeeding a 48-h-starved rat, reaching a peak at 3 h, and then declining slightly (Fig. 1B). Changes in ACC and MCD activity occurred sufficiently early to contribute to these changes. Thus the activity of ACC1, the major isoform in liver, was
increased ~8-fold at 1 h and 10-fold at 3 h of refeeding, and then showed little change up to 24 h. The abundance of ACC protein (ACC1 plus ACC2) was increased only threefold and fivefold at 1 and 3 h, suggesting that covalent changes (e.g., phosphorylation), as well as alterations in enzyme synthesis or degradation, contributed to the increase in activity at these times (Fig. 2, A and B). In keeping with the increases in malonyl-CoA concentration after 1 and 3 h of refeeding, MCD activity was decreased by 33 and 50% at these times, and it decreased further between 3 and 12 h (Fig. 2C). In contrast to ACC, little change in MCD abundance was observed during the first 12 h of refeeding (Fig. 2D), strongly suggesting that the decrease in MCD activity during this time was due to covalent modification. Interestingly, MCD abundance decreased between 12 and 24 h despite the absence of a change in its activity (Fig. 2D).

The activity of FAS, like that of ACC, increased during refeeding; however, the predominant increases in its activity and abundance occurred later (3–12 h; Fig. 3, A and B) and may have accounted for the modest decrease in hepatic malonyl-CoA during this time period. In contrast to ACC, the increase in FAS abundance (2,000%) was much greater than the 100% increase in activity (Fig. 3B).

**GPAT, DGAT, and glycogen.** In keeping with previous reports (27), mtGPAT activity increased with refeeding. In addition, a borderline increase in microsomal GPAT activity was observed (Fig. 4A). The increment in mtGPAT activity (~100%) was similar to that of FAS; however, it occurred later (18–24 h for GPAT vs. predominantly 3–12 h for FAS). The activity of DGAT, a more distal enzyme in the pathway for glycerolipid synthesis, was similarly increased, although the increase occurred somewhat earlier (3–12 h; Fig. 4B).

Liver glycogen content measured at the end of the fast and 12 and 24 h of refeeding was 38 ± 5, 320 ± 12, and 235 ± 15 μmol/g, respectively (n = 5–6 animals).

**DISCUSSION**

The shift of lipid metabolism in liver from catabolism to anabolism in a starved-refed rat is well documented (28, 30). The results of the present study add MCD and DGAT to the list of hepatic enzymes whose activity is acutely or subacutely altered by refeeding. In addition, they raise the possibility that AMPK coordinates the changes in these and other enzymes of lipid metabolism during the starved-refed transition.

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Fig. 2. Activity and protein abundance of acetyl-CoA carboxylase (ACC; A and B) and malonyl-CoA decarboxylase (MCD; C and D) in liver of rats starved for 48 h and then refed a chow diet for the indicated times. Results are means ± SE for 5–6 rats/group. *P < 0.05, #P < 0.01, ##P < 0.001 compared with fasted rats.
In keeping with earlier findings (2, 49), the activity of ACC in liver increased dramatically in the first 1–3 h after refeeding, and this coincided with changes in malonyl-CoA concentration. A novel finding was that MCD activity was diminished by 35 and 50% after 1 and 3 h of refeeding, respectively, and by 80% at 12 h, suggesting that changes in the activity of both enzymes contributed to the increase in whole tissue malonyl-CoA. Because the liver contains two isoforms of ACC (1, 33) and perhaps as many as three isoforms of MCD (E. Joly, N. Ruderman, M. Prentki, unpublished observations), further studies are needed to determine whether all of these isoforms and the pools of malonyl-CoA that they regulate are equally altered during refeeding.

The changes in both ACC and MCD activity during refeeding appeared to occur by more than one mechanism. In agreement with the findings of others, ACC activity was increased at 1 h, due in large part to increases in its specific activity (presumably the result of dephosphorylation) (48) and at later times (1–12 h) due to an increase in abundance (2, 28, 47). A decrease in liver MCD activity, attributable to a change in its abundance, has been reported by Dyck et al. (10) in 48-h-starved rats after 72 h of refeeding. In contrast, in the present study, prior to 12 h of refeeding, decreases in MCD activity were not associated with a change in abundance, suggesting regulation of its activity by dephosphorylation or some other form of covalent modification. A significant decrease in MCD abundance did occur between 12 and 24 h of refeeding; however, it was not accompanied by a change in activity. Increases in FAS, GPAT, and DGAT activity were in general later-occurring events. FAS activation (100%) occurred predominantly between 3 and 12 h of refeeding and was accompanied by a nearly 2,000% increase in its abundance, in keeping with changes of a similar magnitude in FAS mRNA found by many laboratories (28, 37). The observed 100% increases in DGAT and mtGPAT activity, and smaller increases in endoplasmic reticulum-associated GPAT, were observed only after 12 and 24 h. Because of this and the association of these changes in activity with increases in the mRNA of these enzymes (17, 32), they presumably reflect increases in abundance. On the basis of the existing literature, three distinct mechanisms probably explain these findings: 1) covalent modification of ACC (15, 49) and MCD (42) by phosphorylation, which could account for the early changes in their activity; 2) regulation of the abundance of ACC, FAS, and GPAT by the transcriptional activator SREBP-1, the expression of which has been shown to decrease with starvation and increase with refeeding (17, 28) in a time frame similar to that for the abundance of these enzymes; and 3) Regulation of the abundance of MCD by the transcriptional coactivator peroxisome proliferator-activated receptor (PPAR)γ coactivator-1 (PGC-1), perhaps acting via PPARα (25, 45). In keeping with...
the latter possibility, decreases in PGC1 mRNA and protein abundance have been reported to occur in liver in the fed state (38, 57), and the mRNA of CPT I, which is also under the control of PGC1 and PPARα (26), has been shown to diminish during refeeding after a fast (28, 46).

As already suggested, one factor that could coordinate nearly all of these changes in enzyme activity is AMPK. AMPK activation has been shown to affect the activity in liver of all of the enzymes studied here except DGAT (39). In addition, it has been demonstrated to suppress the expression of SREBP-1c (11, 61) and to increase the expression of PGC-1α, which would secondarily activate PPARα (26, 45). The evidence that a decrease in AMPK activity, such as occurs during refeeding, causes physiologically relevant changes in these enzymes in the opposite direction is less clear. On the one hand, in both incubated rat muscle (18) and cultured Hep G2 hepatoma cells (60), incubation in a high-glucose (25–30 mM) medium for 4 and 24 h, respectively, leads to decreases in both AMPK activity and ACC phosphorylation. Furthermore, in the Hep G2 cells the effect of hyperglycemia on ACC phosphorylation was mimicked by expression of a dominant negative (DN) AMPKα2, and it was inhibited when AMPK activity was increased by incubation with metformin or expression of constitutively active AMPK (60). On the other hand, incubation of primary hepatocytes with 25 mM glucose for 16 h failed to diminish AMPK activity, as it did in Hep G2 cells, and the expression of a DN-AMPKα1 did not increase the mRNA of either FAS or ACC (56). The reason for these differing results and whether AMPKα1 and AMPKα2 differentially affect hepatic lipid metabolism is unclear.

Another conundrum relates to the observed changes in GPAT. Incubation of isolated rat hepatocytes with 5-aminimidazole-4-carboxamide-1-β-d-ribofuranoside has been shown to decrease mtGPAT activity by 20–40% within 20 min (35) and a 50% decrease in hepatic mtGPAT, associated with a twofold increase in AMPK activity, has been observed following the cessation of exercise in rat liver and adipose tissue (36). It has been suggested that these acute decreases in GPAT activity could be due to AMPK-catalyzed phosphorylation of either GPAT or a molecule that regulates its activity (35); however, this has yet to be demonstrated. This question aside, why the decrease in AMPK activity observed here during refeeding was not associated with an acute increase in GPAT activity remains to be determined. The later-occurring increase in GPAT activity seen in the present study has previously been demonstrated to correlate closely with increases in both its abundance (27) and mRNA (17).

The notion that AMPK coordinates the changes in ACC, FAS, and possibly other hepatic enzymes during the starved-fed transition is compatible with earlier studies that linked these changes to increases in plasma insulin and decreases in glucagon (2, 34, 53) (Fig. 5). Thus insulin has been reported to decrease AMPK activity in isolated hepatocytes (55) and glucagon to activate hepatic AMPK (43), possibly by a protein kinase A-induced phosphorylation and activation of the AMPK kinase LKB1 (23). In the context of this discussion, it is noteworthy that starvation is also associated with an inhibition of hepatic protein synthesis that is rapidly reversed by refeeding (58). Because AMPK activation inhibits the mammalian target of rapamycin (mTOR) and factors downstream of mTOR (e.g., p70 S6 kinase) that regulate protein synthesis in liver (23), a decrease in AMPK activity could hypothetically also contribute to the transition of hepatic protein metabolism from catabolism to anabolism during refeeding. The fact that the concentration of glucagon is diminished during carbohydrate refeeding makes this an attractive possibility, as glucagon both inhibits protein synthesis and activates AMPK in liver (23). It could also explain the relative lack of change in AMPK (54), ACC (7, 54), and MCD (7) in skeletal muscle during refeeding after a fast, since glucagon has little if any effect on the muscle cell. Recently, it has been demonstrated that the administration for several days of ghrelin, a hormone released by the stomach during starvation, depresses AMPK in liver and increases the mRNA for ACC and FAS and decreases the mRNA for CPT I (5). Whether decreases in ghrelin, such as occur during refeeding after a fast, influenced the changes observed in the present study, remains to be determined.

A decrease in AMPK activity, accompanied by activation of ACC similar to that reported here, was previously observed in the liver of 48-h-starved-refed 200-g rats by Munday et al. (34), and more recently in rats of similar weight that had been trained to meal feed, when given carbohydrate after 21 h of starvation (46). In contrast, Davies et al. (9) failed to observe a difference in AMPK activity in the liver of 400- to 500-g rats studied in the middle of their light (not eating) and dark (eating) cycles despite activation of ACC during the time they were eating. Similarly, Gonzales et al. (13) found only a modest increase in AMPK activity (20%) in liver of mice fasted for 24 h; however, in this study, ACC activity, as reflected by its phosphorylation, was not altered. The reason(s) for these differing results is not clear. Possible factors include differences in age, species, and duration of the fast and whether

Fig. 5. Hypothetical schema linking a decrease in AMPK activity to the transition from catabolism to anabolism in liver during refeeding after a fast. AMPK is diminished by changes in circulating levels of glucagon and insulin. In addition to altering the level of these hormones, hyperglycemia may directly suppress AMPK activity in the hepatocyte. It is proposed that the decrease in AMPK activity leads to inhibition of fatty acid oxidation and increases in the synthesis of fatty acids, glycerolipids and protein (see text for details). Net effects of a decrease in AMPK activity on other parameters altered in liver during refeeding such as protein degradation, gluconeogenesis, and glycogen synthesis remain to be determined.
the animals ate a large amount of food in a short time or nibbled when they were refed.

The decrease in AMPK activity observed during refeeding after a 48-h fast is a readily reversible physiological occurrence. In contrast, sustained and presumably pathophysiologically decreases in AMPK activity have been observed in tissues of rodents that genetically lack leptin, the leptin receptor (59), or interleukin-6 (21) or have a high plasma level of resistin (4). A common phenotype in these animals appears to be insulin resistance and a predisposition to diabetes and dyslipidemia (3, 39, 40). Interestingly, decreases in hepatic AMPK activity, associated with insulin resistance and changes in ACC and MCD similar to those described during refeeding, have been observed in normal rats during a sustained (5- to 24-h) intravenous infusion of glucose at a high rate (24).

In conclusion, the results indicate that the transition from lipid catabolism to anabolism that occurs in liver during refeeding after a fast involves changes in the activity of MCD and DGAT, as well as ACC, FAS, and GPAT. They also raise the possibility that changes in these enzymes are coordinated by a decrease in AMPK activity. In keeping with this notion, while this paper was in review, Fotretz et al. (12) reported that expression of a constitutively active form of AMPKα2 in liver prevents or markedly attenuates increases in the mRNA of ACC1 and -2, FAS, and GPAT, as well as SREBP-1 and carbohydrate response element-binding protein and enzymes governing glucose transport and glycolysis, in fasted-refed mice. In contrast to the present study, however, these investigators observed no decrease in AMPK activity during refeeding (see above), suggesting, as did an earlier report (9), that factors in addition to a decrease in AMPK activity may modulate the postprandial changes in lipid enzymes.

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