**REVIEW**

**Glucagon, cyclic AMP, and hepatic glucose mobilization: A half-century of uncertainty**

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Abstract

For at least 50 years, the prevailing view has been that the adenylate cyclase (AC)/cyclic AMP (cAMP)/protein kinase A pathway is the predominant signal mediating the hepatic glucose-mobilizing actions of glucagon. A wealth of evidence, however, supports the alternative, that the operative signal most of the time is the phospholipase C (PLC)/inositol-phosphate (IP3)/calcium/calmodulin pathway. The evidence can be summarized as follows: (1) The consensus threshold glucagon concentration for activating AC ex vivo is 100 pM, but the statistical hepatic portal plasma glucagon concentration range, measured by RIA, is between 28 and 60 pM; (2) Within that physiological concentration range, glucagon stimulates the PLC/IP3 pathway and robustly increases glucose output without affecting the AC/cAMP pathway; (3) Activation of a latent, amplified AC/cAMP pathway at concentrations below 60 pM is very unlikely; and (4) Activation of the PLC/IP3 pathway at physiological concentrations produces intracellular effects that are similar to those produced by activation of the AC/cAMP pathway at concentrations above 100 pM, including elevated intracellular calcium and altered activities and expressions of key enzymes involved in glycogenolysis, gluconeogenesis, and glycogen synthesis. Under metabolically stressful conditions, as in the early neonate or exercising adult, plasma glucagon concentrations often exceed 100 pM, recruiting the AC/cAMP pathway and enhancing the activation of PLC/IP3 pathway to boost glucose output, adaptively meeting the elevated systemic glucose demand. Whether the AC/cAMP pathway is consistently activated in starvation or diabetes is not clear. Because the importance of glucagon in the pathogenesis of diabetes is becoming increasingly evident, it is even more urgent now to resolve lingering uncertainties and definitively establish glucagon’s true mechanism of glycemia regulation in health and disease.

1 | INTRODUCTION

The metabolic hormone glucagon stimulates the movement of glucose from the liver into the bloodstream. Its main partner, insulin, has a complementary role, to oppose the hepatic glucose-mobilizing effects of glucagon while promoting the movement of glucose out of the bloodstream into glucose-utilizing tissues such as muscle...
and adipose. When in balance, the two hormones effectively maintain euglycemia under variable nutritional and metabolic conditions (Campbell & Newgard, 2021; Zhang et al., 2019).

The central thesis of this review is that glucagon only activates a “basic” cellular signal in liver most of the time, under normal conditions of physical activity and systemic glucose demand. During episodes of elevated glucose demand, as in the early postnatal period or in the exercising adult, glucagon activates both the basic and a “backup” signal simultaneously, boosting the hepatic contribution to systemic glucose supply. Glucagon activates the backup signal much less consistently in the metabolically stressful conditions of starvation and diabetes. The backup signal was characterized first, in 1971, which helps to explain why it has since been the more extensively studied. Even now, it is regarded by most investigators as the main or even exclusive cellular signal pathway mediating the hepatic glucose-mobilizing effects of glucagon most or all of the time. The discovery of the basic pathway was not published until fifteen years later, but only more recently has it begun to receive the attention that it deserves.

The current model of glucagon’s hepatic glucose-mobilizing actions had its origins in the work of the Nobel laureate Earl Sutherland and his colleagues in the early 1970s.

2 | DISCOVERY OF THE BACKUP SIGNAL

In 1971, the year that he was awarded the Nobel Prize for his discovery and chemical characterization of the first known cellular signal (“second messenger”) molecule, cyclic adenosine-3’,5’-monophosphate (cAMP) (Sutherland, 1972), Earl Sutherland—along with his colleagues John Exton, Al Robison, and Charles Park—published a groundbreaking paper on metabolic effects of glucagon on the liver (Exton, Lewis, et al., 1971). They used glucagon, epinephrine, and norepinephrine as probes to stimulate the production of cAMP and the mobilization of glucose in the isolated perfused rat liver. They calculated that the threshold concentration (TC) of glucagon required to generate measurable levels of cAMP in the tissue was $2 \times 10^{-10} \text{ M (200 pM)}$. Since then, the majority of studies, largely on perfused livers, hepatocytes, or hepatocyte membranes (see below), collectively indicate that the TC is half that, at or near $1 \times 10^{-10} \text{ M (100 pM)}$.

Sutherland and coworkers proposed that concentrations of glucagon in the plasma perfusing the liver need to reach that threshold in order to activate the signal in vivo: “It is important to consider whether the concentrations of glucagon … promoting cyclic AMP accumulation in the isolated liver, lie within the normal range in portal venous blood.” To provide supporting evidence that they do, the authors cited two reports that had been published two years earlier (Buchanan et al., 1969; Ohneda et al., 1969). According to those studies, plasma glucagon levels in the canine pancreatic-duodenal vein ranged between around 200 and 1000 pM. On that basis, they concluded that “…the minimal effective concentration of glucagon for the promotion of hepatic cyclic AMP accumulation and glucose mobilization observed in the present study ($2 \times 10^{-10} \text{ M}$) would not be out of line with the probable level of glucagon in portal venous blood”. That statement, possibly more than any other, has directed the focus on cAMP as the main or exclusive intracellular mediator of glucagon’s actions ever since. In retrospect, however, the plasma glucagon estimates in the reports that they had cited appear to have been erroneously high. A wealth of subsequent studies confirms that glucagon concentrations in the hepatic portal and extrahepatic circulations of adult mammals, under normal conditions, are well below the 100 pM TC required to activate adenylate cyclase (AC), enhance the production of cAMP, and activate protein kinase A (PKA) in perfused livers, hepatocytes, and hepatocyte membranes (Rodgers, 2012).

A fundamental and persistent problem with the current model, rarely acknowledged or addressed, is that there is not enough glucagon in the hepatic portal circulation to activate AC in liver most of the time.

3 | PLASMA GLUCAGON CONCENTRATIONS

In the two reports of hepatic portal plasma glucagon concentrations cited by Sutherland and colleagues, the authors used radioimmunoassay (RIA) techniques to arrive at their estimates. But RIA-based methods, especially those that were applied in the 1960’s, were generally not optimal in terms of accuracy, specificity, and sensitivity (Aguilar-Parada et al., 1969; Bak et al., 2014; Wewer Albrechtsen et al., 2014; Wewer Albrechtsen, Kuhre, Pedersen, et al., 2016; Wewer Albrechtsen, Kuhre, Windeløv, et al., 2016; Wewer Albrechtsen, Veedfald, et al., 2016). Peptides whose sequences partially overlap that of glucagon, including glucentin and oxyntomodulin, can interfere, producing erroneously high readings (Bak et al., 2014; Holst, 1983, 2014).
Further, glucagon is subject to degradation in plasma or when stored in the freezer for long periods (Deacon et al., 2003; Wewer Albrechtsen et al., 2015) or when incubated with liver membranes (Baumann et al., 1981), and recovery is not consistently reported. Estimates can also differ depending on the part of the glucagon molecule for which the antibody has affinity (Deacon et al., 2003; Soybel et al., 1983; Trebbien et al., 2004). For example, RIA antibodies directed at the carboxyl, amino, and mid-region of the molecule yielded values of 16, 34, and 58 pM, respectively, in the same plasma sample (Deacon et al., 2003). Largely because they may not rely on the same antibody, various commercial RIA kits can yield estimates that are different from each other and from those produced by in-house RIAs (Bak et al., 2014; Wewer Albrechtsen et al., 2014). As an acknowledgement of uncertainties implicit in the concentration estimates obtained by RIA, the hormone is commonly designated “immunoreactive glucagon”. In recent years, enzyme-linked immunosorbent assays (ELISA) have been gradually replacing standard RIA techniques as the method of choice because they have been demonstrated to be, on the whole, more specific and sensitive, and thus more accurate, than conventional RIAs (Holst, 1983, 2014). Predictably, they also generally yield lower values than RIA techniques do (Ichikawa et al., 2019; Miyachi et al., 2017). Nevertheless, until recently, by far the largest portion of the reported measurements of plasma glucagon levels over the last half-century have been determined by RIA. In the following sections, plasma glucagon concentrations can be assumed to be those in the peripheral venous circulation determined by RIA unless otherwise specified (Figures 1–3, Table 1).

As Sutherland and coworkers proposed, the validity of the cAMP hypothesis – that the AC/cAMP pathway mediates the hepatic glucoregulatory actions of glucagon – is critically dependent on establishing that hepatic portal plasma concentrations of glucagon are sufficient to activate AC in liver. A large body of evidence gathered since then confirms that most of the time they are not. According to 36 reports published between 1976 and 2017, the composite mean hepatic portal plasma glucagon concentration, measured by RIA, in metabolically unstressed, fed or fasted humans, dogs, rats, and pigs at rest, conscious or under anesthesia, is 43.9 ± 3.3 pM (Figure 1), with a 99.9999% confidence interval of 27.8–60.0 pM. Note that all of the individual mean values shown in Figure 1 are below 100 pM, the consensus TC for activating AC ex vivo (see below). According to these results, there is not nearly enough glucagon in hepatic portal plasma—in either anesthetized or conscious animals at rest—to activate AC in liver most of the time. But they can occasionally rise to levels sufficient to activate AC during intervals of elevated metabolic stress (discussed below).

For purposes of this review, plasma glucagon concentrations are divided into four zones (Figure 2a; Coker, Koyama, Brooks, et al., 1999). Categorizing them this way highlights the importance of distinct plasma concentration ranges as they apply to the hormone’s mechanism of action under varying conditions. Zone 1 is between 0 and 60 pM, the statistical hepatic portal glucagon concentration range under normal conditions as described above and in the legend for Figure 1. It can be defined as “physiological glucagonemia,” spanning plasma glucagon concentrations in the largest group, fed or fasting (less than 24 h and usually overnight) healthy adult experimental animals and humans at rest or during normal physical activity. Mean concentrations

**FIGURE 1** Distribution of hepatic portal plasma glucagon concentrations (pM) in fed or fasted humans, dogs, rats, and miniature pigs, measured by radioimmunoassay (RIA). Durations of fasting or starvation were 12–48 h. The 36 values are means determined by RIA between 1976 and 2017. Plasma samples were obtained from fed, fasted, starved, conscious, or anesthetized animals. Twenty of the 36 mean values were obtained using “Unger’s 30K antibody”, with most of the remainder using commercial RIA kits. Statistical values: Composite mean = 43.9 pM; SD = 19.8; SEM = 3.3; and the 99.9999% confidence interval = 16.1, for a statistical range of 27.8 – 60.0 pM.

There were no obvious correlations between plasma concentration and species, nutritional state, or RIA method. Note that all 36 values are below the consensus TC of 100 pM for activating AC (see Figure 4). The 36 sources were: Kraft et al. (2017), Lewis et al. (1997), Saccà et al. (1979) and Vaitkus et al. (1984), (10–19); Berger et al. (1994), Blommaart et al. (1993), Curnow et al. (1976), Fries et al. (1982), Hickman et al. (1992), Imai et al. (2003) and Wasserman et al. (1993) (20–29); Androgé et al. (1985), Baumann et al. (1981), Cersosimo et al. (1989), Rao (1995) and Sherwin et al. (1977) (30–39); Francavilla et al. (1980), Holst et al. (1980), Langhans et al. (1984) and Müller et al. (1984) (40–49); Demigné et al. (1985), Goldstein and Curnow (1978), Horikawa et al. (1998), Jaspan et al. (1984), Silva et al. (1990) and Wolf and Eisenstein (1981), (50–59); Balks and Jungermann (1984), Gannon and Nuttall (1993), Ishida et al. (1981), Kinoshita et al. (1985), McLeod et al. (1983), Rabouit et al. (1989) and Silva et al. (1990) (60–69); Hussein et al. (1986), Latour et al. (1999) and Okuda et al. (1994) (70–79).
Plasma glucagon concentrations as measured by radioimmunoassay (RIA). Mean values ±SEM are grouped by species (a), vascular bed (b), and conditions (c and d), gathered from a large sampling published between 1969 and 2020. (a) Mean peripheral venous plasma concentrations in fed and short-term fasting (less than 24 h) adult humans (H), dogs (D), rats (R), and mice (M). When fasted, the durations were 8–12 h in humans, 13–24 h in dogs, 12–16 h in rats, and 14–16 h in mice. (b) Plasma levels in hepatic portal (HP; see Figure 1), peripheral venous (PV), and arterial (AR) circulations. The mean values in the peripheral venous and arterial circulations are 67% and 32%, respectively, of those in the hepatic portal plasma. (c) Mean peripheral venous plasma glucagon levels in type 1 diabetes (T1D; STZ- or alloxan-induced diabetes in mice and rats), type 2 diabetes or obesity with insulin resistance in humans (T2D), and in starvation (S) of more than 24 h. Durations of starvation were 2.25–6 days in rats and 3–7 days in humans. (d) Mean peripheral venous plasma glucagon levels in neonates (N) and exercising adults (E). The ages of neonates ranged between newborn and 4 days in humans (n = 5) and between newborn and 14 days in mice and rats (n = 8). Durations of exercise in rats, dogs, and humans varied from 1 h to exhaustion. The numbers in parentheses indicate the number of publications from which the data were averaged. Data from some references applied to more than one grouping. Plasma concentrations are divided here into four zones based on RIA estimates: Zone 1 (normal physiological) between 0 and 60 pM, the upper limit of which is the consensus TC for activating AC in dose-response curves generated ex vivo (see text and Figure 4); Zone 2 (physiological hyperglucagonemia) between 100 and 800 pM, includes the highest mean plasma glucagon concentration in neonates (600 pM) (Blommaart et al., 1995) or exercising adults (732 pM) (Seitz et al., 1999) instead of RIA (Hussein et al., 1986). The data were compiled from the following references: A (Species)–Humans (H): Bolli et al. (1984), Borghi et al. (1984), Brodows (1985), Evans et al. (2004), Fujita et al. (1975), Gosmanov et al. (2005), Hamaguchi et al. (1991), Hansen et al. (1982), Heise et al. (2004), (2004), Henkel et al. (2005), Jaspal et al. (2009), Kalkhoff et al. (1973), Livingston et al. (1985), Okba et al. (2020), Petersen and Sullivan (2001), Porcellati et al. (2003), Raju and Cayer (2005), Sherwin et al. (2005), Tasaka (2005), Verillo et al. (1988); Dogs (D): Cersosimo et al. (1998), Coker, Koyama, Brooks, et al. (1999), Kraft et al. (2017), Ishida et al. (1983), Moore et al. (2014), Sherk et al. (2001), Sindelar et al. (1998), and Vaitkus et al. (1984); Rats (R): Balaks and Jungermann (1984), Charbonneau et al. (2005), Langhans et al. (1984), Latour et al. (1999), Mayor and Calle, (1988), Omer et al. (2004), Powell et al. (1993), Ruiter et al. (2003), Unger et al. (1985), Widmaier et al. (1991) and Winzell et al. (2007), Mice (M): Green et al. (2016), Karlsson et al. (2002), Marty et al. (2005), Parker et al. (2002), Perry et al. (2020), Winzell et al. (2007) and Zhang et al. (2018) B (Vascular Bed - Hepatic Portal (HP): See legend, Figure 1, Peripheral Venous (PV): Balaks and Jungermann (1984), Bolli et al. (1984), Cersosimo et al. (1998), Charbonneau et al. (2005), Coker, Koyama, Brooks, et al. (1999), Hamaguchi et al. (1991), Hansen et al. (1982), Heise et al. (2004), Henkel et al. (2005), Ichikawa et al. (2019), Ishida et al. (1981), Jaspal et al. (1984), Karlsson et al. (2002), Langhans et al. (1984), Latour et al. (1999), Livingston et al. (1985), Marty et al. (2005), Mayor and Calle (1988), Nair et al. (1987), Omer et al. (2004), Parker et al. (2002), Perry et al. (2020), Powell et al. (1993), Raju and Cayer (2005), Shi et al. (1996), Wall et al. (2005) and Winder, Arogyasami, et al. (1988), Arterial (AR): Balaks and Jungermann (1984), Carlson and Winder (1999), Coker, Koyama, Brooks, et al. (1999), Jackson et al. (2004), Moore et al. (2014), Patel (1984), Pencek et al. (2004) and Sherck et al. (2001), C (Diabetes or Starvation) - Type 1 Diabetes (T1D): Chamras et al. (1980), Green et al. (2016), Hermida et al. (1994), Mayor and Calle (1988), Meek et al. (2015), Patel (1984), Shi et al. (1996), Srikant et al. (1977), Walsh and Dunbar (1984), Widmaier et al. (1991), Yamashita et al. (1980) and Zhang et al. (2018), Type 2 Diabetes (T2D): Bolli et al. (1984), Borghi et al. (1984), Hamaguchi et al. (1991), Henkel et al. (2005), Knop et al. (2005), Marliss et al. (1970), Nair et al. (1970); Starvation: Aguilar-Parada et al. (1969), Bois-Joyeux et al. (1986), Brodows (1985), Goldstein et al. (1978b), Hamaguchi et al. (1991), Henkel et al. (2005), Knop et al. (2007), Marliss et al. (1970), Mlekusch et al. (1981), Nair et al. (1987), Seitz et al. (1976), Srikant et al. (1977), Vairil et al. (1988), D (Neonates or Exercising Adults) - Neonates (N): Blommaart et al. (1995), Fernández-Milán et al. (2013), Girard et al. (1973), Luycx et al. (1972), Lyonnet et al. (1988), Milner et al. (1972), Movassat et al. (1997), Nurjhan et al. (1985), Ogata et al. (1988), Salle and Ruiton-Ugliengo (1977) and Sperling et al. (1974), Exercise (E): Carlson and Winder (1999), Charbonneau et al. (2005). Coker, Koyama, Lacy, et al. (1999), Latour et al. (1999), Sellers et al. (1988), Winder, Arogyasami, et al. (1988), Winder et al. (1981) and Winder, Yang, et al. (1988)
reported in the literature for this group consistently fall well within zone 1 regardless of species (human, dog, rat, or mouse; Figure 2a) or vascular bed (hepatic portal, peripheral venous, or arterial) (Figure 2b). Mean plasma glucagon concentrations of rabbits, pigs, cats, and cattle are also within zone 1 (20–50 pM) (Brand et al., 1996; Deacon et al., 2003; Kavianipour et al., 2003; Trebbien et al., 2004; Uvnäs-Moberg et al., 1986; Williams et al., 2006). Of course, concentrations in the hepatic portal circulation are higher than those in peripheral vascular beds because the former is between the pancreas and the liver. Note here that, according to the reports cited in Figure 2, mean plasma glucagon concentrations in the hepatic portal circulation are 50% and 140% higher than they are in the peripheral venous and arterial vascular beds respectively (Figure 2b), reflecting the hepatic extraction and distribution of the hormone (Balks & Jungermann, 1984; Dobbins et al., 1995; Ishida et al., 1981; Jaspan et al., 1984; Trebbien et al., 2004). As discussed in more detail below, when circulating glucagon concentrations vary within zone 1, as they do in the metabolically unchallenged mammal at rest (Balks & Jungermann, 1984), hepatic AC activity and tissue cAMP levels are constitutive (basal), and not affected by the hormone.

Zone 2 is between 60 and 100 pM. The upper limit of zone 2 is the consensus TC for AC activation determined ex vivo. The zone 2 concentration range is described here as “transitional” because glucagon at concentrations within that range can stimulate hepatic AC activity, but inconsistently and unpredictably. Mean plasma glucagon concentrations in experimental insulin-dependent diabetics (T1D) or starvation can be in zone 1 or zone 2 (Figure 2c), with variable effects on AC activity. Mean plasma concentrations in human type 2 diabetics (T2D), by contrast, are uniformly within zone 1 (Figure 2c), and therefore would not be expected to activate AC in that condition.

Zone 3, which is categorized here as “physiological hyperglucagonemia,” ranges between 100 and 800 pM. Two groups whose mean plasma glucagon concentrations usually fall into this range are early neonates and exercising adults (Figure 2d). The upper limit of 800 pM was selected to capture

![Graph showing human venous plasma glucagon concentrations measured by RIA, double-antibody sandwich enzyme-linked immunosorbent assay (ELISA), and liquid chromatography/mass spectrometry (LC/MS). Values were obtained from peripheral venous blood samples taken from 13 healthy human volunteers. The authors included sitagliptin, an inhibitor of dipeptidyl peptidase-4, in their assay solution, a precaution not always taken. They did not specify whether they assayed fresh or frozen-thawed samples. Notably, the mean values as assessed by ELISA and LC/MS were 50% and 33%, respectively, of those measured by RIA. The figure is adapted from Miyachi et al. (2017). The data clearly reveal the profound influence of assay technique on estimates of plasma glucagon concentrations.]

**TABLE 1** Comparisons of plasma glucagon concentrations as measured by ELISA and RIA

| Condition       | Plasma glucagon concentration (pM) | ELISA/RIA | Reference          |
|-----------------|-----------------------------------|-----------|--------------------|
| Normal          | ELISA 6                           | RIA 22    | 0.27               | Ichikawa et al. (2019) |
| Prediabetic     | ELISA 10                          | RIA 23    | 0.43               | Ichikawa et al. (2019) |
| Diabetic        | ELISA 13                          | RIA 27    | 0.48               | Ichikawa et al. (2019) |
| Normal          | ELISA 12                          | RIA 24    | 0.50               | Miyachi et al. (2017)  |
| Obese, NGT      | ELISA 13                          | RIA 27    | 0.48               | Wewer Albrechtsen et al. (2014) |
| Mean ±SEM       | 10.8 ± 1.3                        | 24.6 ± 1.0| 0.43 ± 0.04        |                    |

Note: Values are from peripheral venous samples taken from nondiabetic or T2D humans. The mean value as measured by RIA in this group is close to the collective estimates obtained from nondiabetic and T2D humans in Figure 1 A and C: 26 and 27 pM, respectively. According to the ELISA/RIA ratio calculated here of 0.43, the mean concentration in the hepatic portal circulation in fed or short-term fasting nondiabetic adults, as measured by RIA, of 44 pM (Figure 1) would be at or near 19 pM if measured by ELISA, 1/5 of the TC for AC activation, 100 pM, determined ex vivo (Figure 4). NGT, non-glucose-tolerant.
the full range of mean plasma concentrations in those two groups. Among the sources cited here (the citation list is in the legend of Figure 2), the highest reported mean plasma concentration in early neonates was 600 pM (Blommaart et al., 1995), and in exercising adults was 732 pM (Sellers et al., 1988). When plasma glucagon concentrations enter zone 3, they predictably activate the AC/cAMP pathway.

Zone 4, from 800 pM to as high as 1,000,000 pM, can be described as “pharmacological,” achieved in vivo only under extraordinary circumstances or produced by administration of high concentrations ex vivo or high doses of exogenous glucagon in vivo. For example, peak mean plasma concentrations after a dose of 1 mg, administered to severely hypoglycemic diabetic patients, can reach 1,300 pM (Blauw et al., 2015). Examples of conditions or experimental manipulations that result in zone 3 or zone 4 plasma concentrations include: arginine administration in vivo (Aguilar-Parada et al., 1969; Gehrand et al., 2016); pronounced insulin-induced hypoglycemia (Powell et al., 1993); or administration of 2-deoxyglucose (Karlsson et al., 2002). Plasma concentrations are also markedly elevated in the hyperglucagonemia of rare alpha cell tumors (Batcher et al., 2011) or in glucagon receptor knockout mice (Gelling et al., 2003). Notably, in experimental studies of glucagon’s cellular mechanism of action, investigators often expose tissue or cellular preparations to 100,000 pM (usually expressed as 100 nM), an extreme zone 4 pharmacological concentration.

Bear in mind that the limits of these plasma glucagon concentration zones are based on estimates obtained by
RIA, and as such they are very likely overestimates. “In normal physiology, circulating concentrations of glucagon are in the lower picomolar range. In the [usually overnight] fasting state with plasma glucose levels around 5 mmol/L, glucagon is secreted in basal levels resulting in plasma concentrations below 20 pmol/L [italics added]” (Rix et al., 2019). That conclusion was based on results of studies using C-terminal-directed RIA and ELISA-based methods. ELISA assays tend to be more specific and sensitive than conventional RIAs are, and thus tend to yield lower values (Bak et al., 1985; Wewer Albrechtsen et al., 2014; Wewer Albrechtsen, Veedfald, et al., 2016). Peripheral venous plasma glucagon concentrations measured by RIA and ELISA are shown in Figure 3 and Table 1. Miyachi et al. (2017) compared three assay techniques for measuring human plasma glucagon levels: RIA, ELISA, and liquid chromatography/mass spectrometry (LC/MS) (Figure 3). They determined that peripheral venous samples taken from 13 volunteers, subjected to an overnight fast, yielded mean values of 24 pM by RIA, 12 pM by sandwich ELISA, and 8 pM by LC/MS. The ELISA/RIA concentration ratio of 50% is close to the ratio of 43% calculated from direct comparisons carried out in a group of studies listed in Table 1. Thus, the collective mean hepatic portal and peripheral venous plasma glucagon concentrations determined by RIA of 43.9 and 28.8 pM, respectively, as depicted in Figures 1 and 2b, might have been closer to 19 and 12 pM had they been measured by ELISA instead. The last is identical to the value of 12 pM reported for the ELISA-based estimates obtained from peripheral venous plasma reported by Miyachi and coworkers. Peripheral venous plasma values of around 6 pM in the rat were reported by Xue, Cei, et al. (2017) using the Millipore RENDOI-85K rat endocrine Linco-plex assay, and in humans by Kobayashi (Kobayashi et al., 2020) using a sandwich ELISA assay. Based on the hepatic portal/peripheral venous concentration ratio of 1.5 depicted in Figure 2b, mean peripheral venous concentrations of 6–19 pM would translate to 9–29 pM in the hepatic portal circulation. It can thus be tentatively concluded that the mean hepatic portal plasma glucagon concentration in adult mammals including humans, under fed or short-term fasting conditions at rest or during normal physical activity, may be anywhere from 9 to 29% of the AC-activating TC, determined ex vivo, of 100 pM.

Seemingly, if it were any other hormone, such a substantial gap between normal physiological plasma concentrations and those required to generate the signal of interest would be sufficient grounds to rule out that signal as a mediator of the hormone’s effects. Indeed, Sutherland and coworkers, again in their 1971 paper (Exton, Robison, et al., 1971), applied that very logic to all but dismiss in vivo glucose-mobilizing actions of two other hormones they looked at, the catecholamines epinephrine and norepinephrine. Their conjecture has withstood the test of time. The basal plasma epinephrine concentration range in rats or humans at rest is roughly 200–1,500 pM, but can increase 5- to 20-fold during periods of elevated metabolic stress such as strenuous exercise (Carlson et al., 1985; Jean et al., 1991; Sellers et al., 1988; Winder et al., 1979, 1981, 1982), reaching peak levels as high as around 10,000 pM. By contrast, the epinephrine TC required to activate AC in Sutherland’s 1971 report was 28,000 pM. According to more recent reports, the TC can be as high as 100,000 pM (Noguchi et al., 1985; Pilkis & Ghranner, 1992; Pilkis et al., 1975; Yagami, 1995). Thus, even peak mean plasma epinephrine concentrations under stressful conditions are about 10 to 35% of the TC required to activate AC. Similar disparities apply to circulating norepinephrine as well. Sutherland et al. (Exton, Robison, et al., 1971) concluded: “... peak catecholamine concentrations [in dogs and humans] are lower than the minimal levels [TCs] for activation of cyclic AMP accumulation or glycogenolysis in the isolated rat liver...[T]hese data would preclude circulating epinephrine or norepinephrine from physiological roles in the regulation of hepatic glucose output [italics added]”. The authors were likely assuming that cAMP was the exclusive cellular signal mediating the glucose-mobilizing actions of either glucagon or the catecholamines. They were of course unaware of the alternative signal discovered a decade and a half later, one that is activated by lower concentrations of either glucagon or the alpha agonist component of catecholamines (see the next section). Its alpha receptor-activating property (Laville et al., 1987) accounts for the relatively low, physiological TC, 1000 pM, of epinephrine required to increase glucose output from rat hepatocytes (Bizeau & Hazel, 1999). The most important point here is that the disparities between mean plasma concentrations of epinephrine (under stress) or glucagon (under normal conditions) and their respective concentrations required to activate AC ex vivo are comparable, about 3- to 10-fold for epinephrine and around 3- to 11-fold for glucagon.

If that logic, as it applied to catecholamines, was valid for the scientists who discovered the signal, then the question remains: In light of a similar gap between plasma concentrations and those required to activate AC, why don’t most investigators apply the same criterion to glucagon and at least question the prevailing view that the AC/cAMP pathway is the major or exclusive mediator of its hepatic metabolic actions “most or all of the time”? In spite of strong evidence that they would be justified in doing so, most ignore the question altogether. The longstanding cAMP bias persists (e.g. Agius, 2007; Han et al., 2016; Janah et al., 2020; Jitrapakdee, 2012; Miller & Birnbaum, 2016; Wewer Albrechtsen et al., 2019; Yang & Yang, 2016; Zhang et al., 2019), despite the existence of a widely-known,
surprisingly well-characterized, and much more physiologically relevant alternative signal.

The solution to the problem was discovered in 1986: There is more than enough glucagon in hepatic portal plasma to activate an alternative, physiologically relevant signal pathway to increase hepatic glucose mobilization.

4 | DISCOVERY OF THE BASIC SIGNAL

In 1986, fifteen years after Sutherland and coworkers published their seminal report, Miles Houslay and colleagues published another landmark paper on hepatic actions of glucagon, this time on rat hepatocytes (Wakelam et al., 1986). Among their findings were: (1) Between the concentrations of 10 and 3000 pM, glucagon stimulated the production of inositol phosphates from membrane phospholipids dose-dependently; (2) The TC of glucagon required to increase the production of cAMP was above 100 pM; and (3) A derivative of glucagon, 1-N-α-trinitrophenylhistidine-12-homoarginine (TH)-glucagon, also increased the production of inositol phosphates, to a similar extent over a similar concentration range, without affecting cellular levels of cAMP. They proposed that glucagon activates two receptors, which they called GR1, coupled to inositol-phosphate production, and GR2, coupled to AC. The existence of two glucagon binding sites in liver has been confirmed by direct receptor binding studies (Bonnevie-Nielsen & Tager, 1983; Chamras et al., 1980; Ikezawa et al., 1998). It is now known that the high-affinity, low-density GR1 receptor is coupled to phospholipase C (PLC) and enhanced production of the biologically active inositol-3,4,5-triphosphate (IP3) (Goldstein & Hager, 2018; Müller et al., 2017; Rix et al., 2019). The low affinity-high density GR2 receptor, linked to the increased production of cAMP as a product of AC activation (Agius, 2007; Han et al., 2016; Miller & Birnbaum, 2016; Yang & Yang, 2016) is by far the more widely studied.

4.1 | Glucose output produced by activation of the two signal pathways

Studies carried out ex vivo show that glucagon promotes hepatic glucose mobilization by activating either pathway, but does so over different but overlapping concentration ranges, as shown in Figure 4. The figure highlights several observations of importance. It clearly shows the disparity between the statistical physiological hepatic portal plasma concentration range, derived from the data in Figure 1, and the consensus TC for activating AC ex vivo, 100 pM, the result of a composite concentration-effect curve generated from 15 studies published between 1971 and 1995. Within zone 1, the stimulation of glucose mobilization is associated with a submaximal but measurable and concentration-dependent increase in the generation of inositol phosphates, with little or no activation of AC. According to the composite concentration-effect curve generated by combining 11 studies published between 1971 and 1990, physiological, zone 1 glucagon concentrations stimulate glucose mobilization (gluconeogenesis, glycogenolysis, or glucose output) up to about 40% of the maximum, while activating the PLC/IP3 pathway exclusively. In zone 2, glucagon exerts variable effects on AC activity. Across zones 3 and 4, between 100 and 10,000 pM, glucagon maximally activates the PLC/IP3 pathway while sub-maximally activating the AC/cAMP pathway and further increasing glucose mobilization. Maximal activation of the AC/cAMP pathway, with inhibition of the PLC/IP3 pathway, is achieved by high zone 4, pharmacological concentrations (Figure 4).

The dual effects of glucagon are incorporated into a proposed new model. Under normal conditions, glucagon regulates hepatic glucose mobilization exclusively via the PLC/IP3 pathway. During intervals of high systemic glucose demand, such as in the early neonate or strenuously exercising adult, glucagon at higher plasma concentrations boosts hepatic glucose output to meet the elevated glucose demand by activating both the PLC/IP3 and AC/cAMP pathways simultaneously.

Figure 4 clearly reveals the dual nature of glucagon’s glucose-mobilizing actions on the liver. Below 60 pM, the hormone activates GR1 receptors and the PLC/IP3 pathway to stimulate glucose mobilization up to 40% of maximal capacity without activating AC. This corresponds to plasma concentrations that are characteristic of...
the normal, metabolically unstressed condition (Figure 2a and b). Above 100 pM, glucagon further stimulates glucose mobilization by activating both GR1 and GR2 receptors and the PLC/IP3 and AC/cAMP pathways simultaneously, applicable to the mean plasma concentrations found in the early neonate and exercising adult (Figure 2d). This suggests that, most of the time in vivo, glucagon regulates hepatic glucose output up to 40% of maximal capacity via the PLC/IP3 pathway exclusively. As plasma concentrations rise above 100 pM in response to elevations in systemic glucose demand, glucagon concentration-dependently and adaptively boosts glucose output, by robustly activating the PLC/IP3 pathway toward its maximum and by submaximally activating the AC/cAMP pathway.

This dual effect mediated by two signal pathways seems to be a variation on a broader theme, applicable to target tissues other than hepatocytes. At a concentration of 10,000 pM, glucagon increased cAMP in rat renal glomerular mesangial cells (Li et al., 2006). The same concentration also increased calcium mobilization, an effect that was totally blocked by the PLC inhibitor U73122, implicating the PLC/IP3 pathway. At 1000 pM, sufficient to maximally increase prostaglandin production, glucagon markedly increased both cAMP and inositol phosphates in hepatic Kupffer cells (Hespeling et al., 1995). As in hepatocytes, the operative signal in Kupffer cells most of the time is probably the PLC/IP3 pathway alone because the stimulation of prostaglandin production was maximal or near-maximal at 100 pM. Human adipose tissue is nearly or completely unresponsive to lipolytic effects of glucagon, even at zone 4 concentrations (Galsgaard et al., 2019; Rodgers, 2012). Recently, however, (Pereira et al., 2020) reported that glucagon concentration-dependently but weakly enhanced glycerol release by human hepatocytes, with a TC of 10 pM. The effect of that low physiological concentration on glycerol release was statistically significant but one of a similar magnitude on glucose uptake was not because it was too variable. It seems that the physiological receptor and signal mediating effects of glucagon at physiological concentrations on human adipocytes remain to be identified. Glucagon may also activate two signals in the heart (Harney & Rodgers, 2008). In addition to activating the AC/cAMP pathway to increase contractility at supraphysiological concentrations, at or above 300 pM, glucagon at physiological concentrations, between 10 and 80 pM, also substantially enhanced cardiac glucose utilization by interacting with an uncharacterized receptor coupled to PI3-kinase, presumably with only the latter operative in vivo under normal conditions (Harney & Rodgers, 2008; Rodgers, 2012). Although glucagon clearly works against insulin on the liver to promote glucose output, it may also partner with insulin on the heart to stimulate glucose uptake and utilization. In the following sections, hepatic glucose-mobilizing effects of glucagon that are mediated exclusively by activation of GR1 receptors and the PLC/IP3 pathway at physiological concentrations are presented, along with examples of overlapping cellular responses to activation of the AC/cAMP pathway by higher concentrations.

The two intracellular signal pathways activated by glucagon in hepatocytes are largely redundant. Many of the downstream targets of the PLC/IP3 pathway are the same as those of the AC/cAMP pathway.

5 | INTRACELLULAR EFFECTS OF ACTIVATING THE PLC/IP3 signal

Activation of the PLC/IP3 pathway at physiological concentrations generates many of the same intracellular effects as does the activation of the AC/cAMP pathway at supraphysiological and pharmacological concentrations. Downstream targets of the two pathways overlap extensively. Common intracellular effects include increases in calcium concentrations and altered activities and expressions of the same enzymes. In the following discussion, downstream targets of the PLC/IP3 pathway are described in some detail, along with examples of similar or identical targets or effects of activating the AC/cAMP pathway (Table 2, Figures 4 and 5).

5.1 | Signal activation and calcium mobilization

Activation of either glucagon GPCR isoform increases intracellular calcium (Ca^{2+}), but the mechanisms are not identical. The two glucagon GPCRs in liver, GR1 and GR2, are coupled to the G protein isoforms G<sub>q</sub> and G<sub>s</sub>, respectively (Christophe, 1995). Over a broad concentration range (Figure 4), glucagon binds to the high-affinity, low density GR1 GPCR (Andersson et al., 1993; Bonnevie-Nielsen & Tager, 1983; Chamras et al., 1980; Ikezawa et al., 1998) and activates PLC and the formation of phospholipid products including the biologically active inositol-1,4,5-triphosphate (IP3) (Hansen et al., 1982; Unson et al., 1989; Wakelam et al., 1986; Whipp et al., 1987). Binding of IP3 to inositol triphosphate receptors (IP3Rs) releases reticular calcium while generating calcium spikes and oscillations (slower waves) that propagate throughout the cell and increase the total Ca^{2+} levels in the cytosol and
There are two isoforms of the IP3R in hepatocytes, IP3R-I and -II (Hernandez et al., 2007; Hirata et al., 2002; Mauger et al., 1989). Both are located on reticular elements within the cytosol, but the distribution of the IP3R-I receptor is greater, extending into the nucleus (Feriod et al., 2014; Hirata et al., 2002; Klein & Malviya, 2008). IP3R-II

### TABLE 2

| Response                                      | TC or Effective concentration (pM) | References                                                                 |
|-----------------------------------------------|-----------------------------------|---------------------------------------------------------------------------|
| **A. Signal activation and calcium mobilization** |                                   |                                                                           |
| ↑ PLC and inositol-P production               | 5                                 | Wakelam et al. (1986)                                                     |
| ↑ Ca\(^{2+}\) or spike frequency              | 17                                | Berglund et al. (2009), Kass et al. (1994), Mine et al. (1988), Sistare et al. (1985), Somogyi et al. (1992) and Staddon and Hansford (1989) |
| **B. Cytosolic enzyme targets**               |                                   |                                                                           |
| ↑ GPase activity                              | 13                                | Blackmore and Exton (1986), Lynch et al. (1989), Marks and Parker Botelho (1986), Rothermel, Perillo, et al. (1984) and Shiota et al. (1996) |
| ↑ F–1,6-BP phosphorylation                    | 10                                | Aggarwal et al. (1995)                                                   |
| ↑ F–1,6-BP activity                           | 6                                 | Caruana et al. (1981) and Ekdahl and Ekman (1987)                         |
| ↑ F–2,6-BP activity                           | 5                                 | El-Maghrabi et al. (1982)                                                |
| ↓ 6-PF–2K activity                            | 5                                 | El-Maghrabi et al. (1982)                                                |
| ↑ PyrK phosphorylation                        | 10                                | Aggarwal et al. (1995)                                                   |
| ↓ PyrK activity                               | 40                                | Blair et al. (1976), El-Maghrabi et al. (1982), Feliu et al. (1976)       |
| ↑ GS phosphorylation                          | 1                                 | Aggarwal et al. (1995)                                                   |
| ↓ GS activity                                 | 6                                 | Marks and Parker Botelho (1986) and Rothermel, Jastor, et al. (1984)     |
| ↑ AMPK phosphorylation                        | 47                                | Kraft et al. (2017) and Rivera et al. (2010)                             |
| ↑ ACC phosphorylation                         | 57                                | Rivera et al. (2010)                                                     |
| **C. Nuclear targets**                        |                                   |                                                                           |
| ↑ PFKFB1 phosphorylation                      | 10                                | Miller et al. (2013)                                                     |
| ↑ Fox01 phosphorylation                       | 57                                | Rivera et al. (2010)                                                     |
| ↑ CREB phosphorylation                        | 10                                | Miller et al. (2013)                                                     |
| ↑ PEPCK expression                            | 1                                 | Fleig et al. (1984)                                                      |
| ↓ GK expression                               | 34                                | Kraft et al. (2017)                                                      |
| **D. Net responses**                          |                                   |                                                                           |
| ↓ Glycogen synthesis                          | 34                                | Kraft et al. (2017)                                                      |
| ↑ Glycogenolysis                              | 9                                 | Bizeau and Hazel (1999; Corvera and García-Sáinz (1984), Exton, Lewis, et al. (1971), Khan et al. (1980), Rothermel, Jastor, et al. (1984), Yamatani et al. (1987) |
| ↑ Gluconeogenesis                             | 23                                | Exton, Lewis, et al. (1971), Feliu et al. (1976), Fleig et al. (1984) and Shiota et al. (1996) |
| ↑ Glucose output                              | 20                                | Fleig et al. (1984) and Shiota et al. (1996)                             |

**Note:** The TCs were determined from dose-response curves generated in hepatocytes or perfused livers. Effective single concentrations (bold) were achieved by infusions of exogenous hormone in vivo (dogs or mice). The values for F-2,6-BP and 6-PF-2K activities are estimates based on interpolations of the dose-response curves. Where more than one reference is cited for a given response, the indicated TC is the group average. Calculation of the mean was restricted to the TCs determined from dose-response curves ex vivo; single plasma concentrations generated by infusions in vivo were not included.

Abbreviations: ACC, acetyl CoA carboxylase; AMPK, AMP-activated protein kinase; F-1,6-BP, fructose-1,6-bisphosphatase; F-2,6-BP, fructose-2,6-bisphosphatase; 6-PF-1K, 6-phosphofructo-1-kinase; GK, glucokinase; GPase, glycogen phosphorylase; GS, glycogen synthase; PEPCK, phosphoenolpyruvate carboxykinase; PFK, phosphofructokinase; PK, pyruvate kinase.
receptors are concentrated proximal to the canalicular membrane. Increases in Ca^{2+}_{i} produced by GR1 activation then bind to CaM, forming a Ca^{2+}/CaM complex. The complex binds to and activates multiple isoforms of Ca^{2+}/CaM-dependent protein kinases (collectively labelled here as CaMK). CaMKs implicated in the glucose-mobilizing actions of glucagon in liver include CaMKII, CaMKIV, and CaMKKβ (aka CaMKK2) (Cohen et al., 2015; Hook & Means, 2001; Johannessen & Moens, 2007; Shaywitz & Greenberg, 1999; Skelding & Rostas, 2020; Soderling, 1999; Takemoto-Kimura et al., 2017). Apparently, only the IP3R-I isoform mediates the effects of calcium-mobilizing hormones such as vasopressin (Hernandez et al., 2007) or, presumably, glucagon via GR1 activation to increase Ca^{2+}_{i} and generate related downstream effects. Liver-specific knockout of the IP3R-I receptor prevented the increase in CaMKII phosphorylation and other responses to high concentrations of glucagon (Perry et al., 2020). Influences on physiological concentrations were not tested. Knockout of the IP3R-II receptor had little to no effect on glycemia, gluconeogenesis, fasting-induced activation of CaMKII, increases in the expressions of the gluconeogenic enzymes phosphoenolpyruvate carboxykinase (PEPCK) or glucose-6-phosphatase (G6Pase) in response to 50,000 pM glucagon, or on the locations or densities of IP3R-I receptors (Périod et al., 2014).

Mobilization of intracellular calcium is strongly affected by physiological concentrations of glucagon. The
TC required to increase Ca\(^{2+}\) in hepatocytes is most often reported to be 10 pM, a low zone 1 concentration (Kass et al., 1994; Sistare et al., 1985; Staddon & Hansford, 1989). Within zone 1, activation of the PLC/IP3/IP3R pathway by glucagon increases Ca\(^{2+}\), up to approximately 55% of the peak response generated by maximal concentrations of the hormone (Figure 5a). The ability of 50 nM glucagon to produce calcium waves and increase Ca\(^{2+}\) was completely blocked by the PLC antagonist U73312 (Aromataris et al., 2006). Like low concentrations of glucagon, the calcium-mobilizing hormones angiotensin, vasopressin, or noradrenaline increase Ca\(^{2+}\) and induce calcium waves by activating PLC without activating AC (Charest et al., 1985; Hernandez et al., 2007; Pittner & Spitzer, 1993; Ubl et al., 1994; Woods et al., 1986). In the presence of sub-TCs of the alpha agonist phenylephrine, glucagon evoked calcium spikes or oscillations (waves superimposed on repetitive spikes) in rat hepatocytes at concentrations as low as 4 pM (Somogyi et al., 1992), suggesting that the effects of the two hormones are additive.

The dual nature of glucagon’s hepatic effects is represented well by its influence on intracellular calcium. In addition to activating the PLC/IP3 pathway, activating the AC/cAMP pathway also increases Ca\(^{2+}\), but by a slightly different mechanism. At concentrations within zone 3 or higher, sufficient to consistently activate PKA, glucagon adds to the IP3-induced increase in Ca\(^{2+}\) by stimulating PKA-mediated phosphorylation of both reticular IP3Rs and plasma membrane calcium channels (Andersson et al., 1993; Joseph & Ryan, 1993; Wang et al., 2012; Williamson et al., 1987). Thus, at physiological concentrations, glucagon mimics the effects of calcium-mobilizing hormones to increase Ca\(^{2+}\), by activating the GR1/PLC/IP3/CaM pathway exclusively, promoting Ca\(^{2+}\) movement into the cytosol by increasing the binding of IP3 to reticular IP3Rs. At supraphysiological concentrations glucagon further increases Ca\(^{2+}\) by activating both the GR1/PLC/IP3 and GR2/AC/cAMP/PKA pathways simultaneously, the latter adding to the movement of calcium into the cytosol by PKA-mediated phosphorylation of reticular IP3Rs and plasma membrane calcium channels.

Both pathways also seem to be involved in the control of ion flux across the plasma membrane. For example, infusion of anti-glucagon antibodies diminishes chronic hyperpolarizing actions of endogenous glucagon on the liver (Lutz et al., 2001). The hyperpolarizing effect of 100,000 pM glucagon in hepatocytes is inhibited approximately 60% by 1 \(\mu\)M U73122 (Fischer et al., 2005). The residual response in the presence of the high PLC blocker concentration is consistent with the view that alterations of plasmalemmal ion channel conductance are produced by activation of either pathway. Specific plasma membrane ion channels that are targeted by physiological concentrations of glucagon apparently remain to be identified, but both calcium and chloride channels are implicated. At low extracellular calcium, glucagon concentration-dependently promoted net efflux of calcium, at or above 10 pM (Mauger et al., 1989). Vasopressin (10,000 pM) produced a similar response. However, when extracellular calcium concentration is in the normal range, glucagon (10–100 pM) promotes its influx and adds to analogous effects of epinephrine, presumably the alpha agonist component (Poggioli et al., 1986). Activation of plasmalemmal chloride channels by 50,000 pM glucagon was completely blocked by 4 \(\mu\)M U73122 (Aromataris et al., 2006).

Because both glucagon—at physiological concentrations—and the calcium-mobilizing hormones (e.g., noradrenaline, vasopressin, and angiotensin II) act via the GR1/PLC/IP3 pathway, their effects on glucose mobilization should be similar or identical. However, comparisons are usually made using high concentrations of both calcium-mobilizing hormones and glucagon, which presumably activate the PLC/IP3 pathway exclusively regardless of concentration, and glucagon, which activates both the PLC/IP3 and AC/cAMP pathways simultaneously at high concentrations. As a result, the actions of calcium-mobilizing hormones and glucagon, at higher concentrations at least, are often different. Common downstream targets of, and cross-talk between, the overlapping pathways complicates the interpretation of effects of high glucagon concentrations (Goldstein & Hager, 2018; Jiang & Zhang, 2003; Müller et al., 2017; Rix et al., 2019). For example, at their respective maximal concentrations, glucagon (570,000 pM) tripled the basal activity of glycogen phosphorylase (GPase), but vasopressin (200,000 pM), angiotensin II (100,000 pM), or phenylephrine (1,000,000 pM) only increased it 2–2.5-fold in human hepatocytes (Keppens et al., 1993). The differences in this case can be at least partially explained by the ability of higher glucagon concentrations to activate the reticular IP3R two ways as discussed above, one by IP3 binding—like the effects of the calcium-mobilizing hormones—and a supplementary effect produced by PKA-mediated phosphorylation of the IP3R. Other examples of pathway crosstalk points include: CaMKK and CaMKII, which are activated by physiological glucagon concentrations via the PLC/IP3 pathway but can also be phosphorylated by PKA at higher concentrations, inhibiting the activity of the former and stimulating the activity of the latter (Ozcan et al., 2012; Racicopp & Means, 2012; Skelding & Rostas, 2020); AC, which can be inhibited by CaMK (Soderling, 1999); and, as discussed below, PhosK, which can be activated either by elevations in Ca\(^{2+}\) (as a result of activation of either pathway) or by PKA-mediated phosphorylation of the enzyme.

To complicate the picture further, the hepatic effects of calcium-mobilizing hormones are subtly different from...
each other (Kleineke & Söling, 1987) as well as from those of physiological concentrations of glucagon, even though all of these agonists presumably act via the same intracellular signal pathway. Differences are revealed by variations in effects of phorbol ester pretreatment and, in a mechanistically related phenomenon, the extent and kinetics of rapid homologous desensitization. Activation of the PLC/IP3 pathway can generate diacylglycerol (DAG), which binds to and activates PKC, an effect mimicked by phorbol esters (Newton, 2018; Nishizuka, 1989; Püschel et al., 1993). Activated PKC suppresses inositol-phosphate generation by directly inhibiting PLC and by increasing the activity of inositol-phosphate phosphatase (Higashi & Hoek, 1991; Savage et al., 1995; Williamson et al., 1987; Wu-Zhang & Newton, 2013), exerting feedback inhibition of IP3 generation. This mechanism can explain both inhibitory effects of phorbol esters and rapid homologous desensitization exhibited by some—but not all—activators of the PLC/IP3 pathway. Phorbol esters have been reported to inhibit the glucose-mobilizing actions of alpha agonists, but not of vasopressin or angiotensin II (Corvera & Garcia-Sáinz, 1984). Pretreatment with the phorbol ester PMA inhibited the stimulation of GPase by the alpha-agonist component of epinephrine and by 10–1000 pM vasopressin, but did not affect the dose-dependent activation of the enzyme by 10–1000 pM glucagon in rat hepatocytes (Lynch et al., 1985). As predicted, pretreatment with PMA suppressed the increase in glucose output from perfused rat livers produced by norepinephrine but it did not alter the response to 10,000 pM glucagon (Püschel et al., 1993). In rat hepatocytes, the generation of inositol phosphates or the stimulation of GPase by vasopressin faded within 10 min (Hughes et al., 1984), presumably as a result of DAG-induced activation of PKC. However, either glucagon or TH-glucagon (the selective GR1 agonist) produced a sustained increase in inositol-phosphate generation over that same time period (Wakelam et al., 1986), suggesting that the PLC/IP3-dependent pathway activated by glucagon via the GR-1 receptor is not subject to rapid DAG-mediated desensitization. Disparate responses may be explained, at least partially, by differences in G protein isoforms coupled to their receptors or in fatty acyl groups bound to the DAG molecules generated by the different agonists (Hughes et al., 1984; Morel et al., 1992; Wu-Zhang & Newton, 2013; Xu & Xie, 2009).

5.2 Cytosolic metabolic enzyme targets

Activation of the PLC/IP3 pathway alters activities of key cytosolic and mitochondrial enzymes involved in the regulation of glucose metabolism and mobilization (Table 2B), many of which are also altered in the same direction by PKA. Important direct and indirect targets are phosphorylase kinase (PhosK), glycogen phosphorylase (GPase), fructose-1,6-bisphosphatase (F-1,6-BPase), fructose-2,6 bisphosphatase (F-2,6-BPase), pyruvate kinase (PyrK), phosphofructokinase-1 (PFK-1), glycogen synthase (GS), and 5’-AMP-activated protein kinase (AMPK). Activation of either the PLC/IP3/CaM or the AC/cAMP/PKA pathway generally targets the same enzymes and alters their activities in the same direction, but there are differences in detail (Table 2B).

Another good example of the dual action of glucagon is the control of the activity of PhosK, which phosphorylates and activates GPase (Brushia & Walsh, 1999; Miller & Birnbaum, 2016; Rothermel et al., 1984; Shiota et al., 1996), promoting glycogenolysis. PhosK is activated by elevations in Ca\(^{2+}\), which as mentioned earlier can be produced by glucagon in response to activation of either pathway (Roskoski, 2015). At physiological concentrations glucagon increases Ca\(^{2+}\) by activating the GR1/PLC/IP3/CaM pathway, promoting IP3 binding to reticular IP3Rs and the movement of Ca\(^{2+}\) into the cytosol. In addition, at supraphysiological concentrations glucagon can further increase Ca\(^{2+}\) by direct PKA-mediated phosphorylation of IP3Rs and of plasmalemmal calcium transporters in response to the activation of GR2 receptors (Roskoski, 2015). Elevations in Ca\(^{2+}\) produced by either mechanism activate PhosK by increasing the extent of calcium’s binding to the CaM component of the enzyme (Vénien-Bryan et al., 2002). Activated PhosK then converts GPase a to the active GPase b by catalyzing its phosphorylation. The end result of the activation of either pathway is increased glycogenolysis and enhancement of glucose output. One implication is that, in vivo, variations in plasma glucagon within the physiological range would regulate hepatic glycogenolysis at sub-maximal levels by activating GR1 receptors exclusively. Higher plasma concentrations could then enhance the stimulation of glycogenolysis by activating both GR1 and GR2 receptors.

There are other examples of overlap between the pathways with regard to common target enzymes. Either CaMK or PKA catalyzes the phosphorylation of the bifunctional enzyme 6-phosphofructo-2-kinase (6-PFK-2K or PFK2)/fructose-2,6-bisphosphatase (F-2,6-BPase), increasing the activity of the phosphatase component (Brushia & Walsh, 1999; El-Maghrabi et al., 1982; Miller & Birnbaum, 2016; Okar et al., 2004; Ravnskjaer et al., 2015). This would decrease levels of the allosteric regulator fructose-2,6-bisphosphate (F-2,6-BP). In the classical view, declines in F-2,6-BP would lift inhibition of the gluconeogenic enzyme FBP-1 and decrease the activation of the glycolytic enzyme PFK-1, promoting gluconeogenesis while inhibiting glycolytic flux (El-Maghrabi et al., 1982; Exton, 1987; Furuya et al., 1982; Mlekusch...
et al., 1981; Müller et al., 2017; Okar et al., 2004). In addition, either CaMK or PKA can phosphorylate the gluconeogenic enzyme F-1,6-BPase in vitro (Mlekusch et al., 1981) or in hepatocytes, increasing its activity (Casteleijn et al., 1986; Ekdahl & Ekman, 1987; El-Maghrabi et al., 1982). Activation of either pathway can phosphorylate and decrease the activity of the glycolytic enzyme PyrK (Blair et al., 1976; Connelly et al., 1987; Felitu et al., 1976; Mlekusch et al., 1981; Miller & Birnbaum, 2016; Staddon & Hansford, 1989), providing an additional mechanism of promoting gluconeogenesis and inhibiting glycolysis. Glycogen synthase (GS) is a promiscuous target. It can be phosphorylated and inhibited not only by PKA or CaMK, but also by PhosK, PKC, and glycogen synthase kinase, among others (Aggarwal et al., 1995; Camici et al., 1984; Ciudad et al., 1984; Imazu et al., 1984; Juhl et al., 1983; Marks & Parker Botelho, 1986; Rothermel et al., 1984; Staddon & Hansford, 1989; Wang et al., 1986).

Adenosine monophosphate-activated protein kinase (AMPK) is an important metabolic sensor that responds to variations in intracellular AMP/ATP ratios and other regulators to balance anabolic and catabolic cellular processes (Aw et al., 2014; Berglund et al., 2009; Fullerton, 2016; Mihaylova & Shaw, 2011; Salminen et al., 2016; Zhang, Yang, et al., 2019). Glucagon affects its activity exclusively via the PLC/IP3 pathway. Activated CaMKβ, but not PKA, catalyzes the phosphorylation and activation of AMPK (Fujiwara et al., 2016; Fullerton, 2016; Racipppi & Means, 2012; Witters et al., 2006). However, it is not clear how or even whether phosphorylation and activation of AMPK via CaMKβ contributes to the resulting increase in glucose output produced by physiological concentrations of glucagon. The net response may depend on such attendant influences as actions of other hormones and regulators, net flux through multiple enzyme pathways, indirect influences on glucose metabolism imposed by AMPK-mediated alterations in fatty acid metabolism, and net changes in the cellular AMP/ATP ratio and energy state (Aw et al., 2014; Berglund et al., 2009; Fullerton, 2016; Hasenour et al., 2013; Marcelo et al., 2016; Mihaylova & Shaw, 2011; Perry et al., 2020; Willows et al., 2017).

Activated AMPK is often depicted as an inhibitor of gluconeogenesis (Mihaylova & Shaw, 2011). Liver-specific overexpression of AMPK was reported to inhibit glucose output, resulting in hypoglycemia (Foretz et al., 2019). Phosphorylation of CRTC2 by AMPK suppresses translocation of CRTC2 to the nucleus and, by that mechanism, inhibits gluconeogenesis (Yoshida et al., 2019). Pharmacological activation of AMPK in hepatoma cells mimicked effects of insulin to decrease the expressions of the gluconeogenic enzymes PEPCK and G6Pase (Lochhead et al., 2000). Constitutively active AMPKα1 decreased PEPCK expression, while dominant negative AMPKα1 increased PEPCK expression in mouse liver (Viana et al., 2006).

But other results suggest that, under some circumstances, activation of AMPK may contribute to the stimulation of gluconeogenesis and glucose output by glucagon. Starvation can increase plasma glucagon (Figure 2c) and has been reported to increase AMPK activity while also increasing gluconeogenesis and decreasing glycogen synthesis (Munday et al., 1991; Sugden et al., 1993). Hepatocytes from CaMKβ knockout mice exhibited decreased expressions of G6Pase and PEPCK and released approximately 50% less glucose when compared to hepatocytes from wild type mice (Anderson et al., 2012). Exercising rats displayed increases in plasma glucagon (from 19 to 49 pM), hepatic AMPK activity (by 73%), and plasma glucose (by 17%) (Carlson & Winder, 1999).

A substrate of AMPK is acetyl CoA carboxylase (ACC), which catalyzes the conversion of acetyl CoA to malonyl CoA, an intermediate in the lipogenic pathway (Hasenour et al., 2013; McGarry & Brown, 1997). ACC is phosphorylated and inhibited by activated AMPK. The inactivation of ACC by a high concentration of glucagon (100,000 pM) was reported to be independent of PKA, but instead was presumably the result of activation of CaMKKβ and AMPK (Sim & Hardie, 1988). The increase in AMPK activity in exercising rats (Carlson & Winder, 1999) was associated with a reduction of ACC activity of about 66%. Activation of the PLC/IP3/CaM pathway by glucagon has also been proposed to enhance gluconeogenesis indirectly by promoting lipolysis and mitochondrial β oxidation of fatty acids (Perry et al., 2020).

### 5.3 Nuclear effects

The dual actions of glucagon are also represented by its concentration-dependent transcriptional effects (Table 2C). PEPCK catalyzes the conversion of oxaloacetate to phosphoenolpyruvate, a rate-limiting step in gluconeogenesis. G6Pase is the final step in the gluconeogenic pathway, catalyzing the dephosphorylation of glucose-6-phosphate, allowing the export glucose from the liver to the bloodstream (Warner et al., 2020). Indirect and direct evidence suggest that either activation of the PLC/IP3/CaM pathway at physiological concentrations or the simultaneous activation of the PLC/IP3/CaM and AC/cAMP/PKA pathways at higher concentrations increases hepatic gluconeogenesis and glucose mobilization to a great extent by CaMK- or PKA-dependent stimulation of the expressions and thus the activities PEPCK and G6Pase (Barthel & Schmoll, 2003; Hansen & Reshef, 1997; Johannessen & Moens, 2007). Key signal targets and coregulators involved in the transcriptional regulation

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**Table 2C.** Concentration-dependent transcriptional effects of glucagon.

| Concentration (pM) | PEPCK expression | G6Pase expression |
|--------------------|------------------|------------------|
| 1                  | Decreased        | Decreased        |
| 10                 | Decreased        | Decreased        |
| 100                | Decreased        | Decreased        |
| 1000               | Decreased        | Decreased        |
| 10,000             | Decreased        | Decreased        |

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**Figure 2c.** Plasma glucagon levels in exercising rats.
of gluconeogenic gene expression include cyclic AMP response element binding protein (CREB), forhead box class 01 element (Fox01), CRTC2 (aka TORC2), and peroxisome proliferator-activated receptor-gamma co-activator alpha (PGC-1α) (Oh et al., 2013; Zhang, Yang, et al., 2019). Phosphorylation of CREB and Fox01, and dephosphorylation of CRTC2, enhance the expressions of G6Pase and PEPCK, promoting gluconeogenesis (Anyamaneeratch et al., 2015; Li et al., 2019; Oh et al., 2013; Ozcan et al., 2012; Servillo et al., 2002; Wang et al., 2009). CREB can be phosphorylated by CaMKII, CaMKIV, or PKA in vitro (Shaywitz & Greenberg, 1999). CaMKII, CaMKIV, and PKA can all catalyze the phosphorylation of CREB at the ser-133 residue of the kinase-inducible domain, but CaMKII can also phosphorylate it at ser 142, 143, and 156 (Johannessen & Moens, 2007; Shaywitz & Greenberg, 1999). Thus, activation of either pathway by glucagon can theoretically promote phosphorylation of CREB via activation of CaMKII and CaMKIV at physiological concentrations or of CaMKII, CaMKIV, and PKA at supraphysiological concentrations in intact cells (Johannessen & Moens, 2007; Koo et al., 2005; Ravnskjær et al., 2015). Interestingly, in the nucleus CaMKIV is more potent than CaMKII in phosphorylating CREB (Sun et al., 1994), and apparently can phosphorylate and activate CREB as effectively as PKA does (Soderling, 1999) (Table 2C).

Direct and indirect evidence indicate that physiological, zone 1 concentrations influence PEPCK expression via the PLC/IP3 pathway. In mouse liver, exogenous glucagon increased phosphorylation and activation of CREB at an apparent TC of 30 pM (Miller et al., 2013), most likely by ultimately activating CaMKIV (Cohen et al., 2015; Liu et al., 2020). Injection of glucagon (5 μg/Kg) into mice increased CREB phosphorylation, but neither the phosphorylation site nor the effect of the injection on plasma glucagon levels was specified (Koo et al., 2005). Ozcan et al., 2012 reported that fasting-induced activation P38 and CaMKII increased phosphorylation of Fox01 and its subsequent migration to the nucleus, but did not measure parallel changes in plasma glucagon levels. Even against a background of sustained plasma insulin levels, increasing plasma glucagon concentration within zone 1, from around 13 to between 40 and 57 pM, by exogenous glucagon infusion in overnight-fasted dogs increased Fox01 and CREB phosphorylation and PEPCK expression, while decreasing liver glycogen content, glucose incorporation into glycogen, and liver glucose uptake (Kraft et al., 2017; Rivera et al., 2010).

Regulation by glucagon of G6Pase expression at physiological concentrations in vivo is not as clearly defined as it is for higher concentrations ex vivo, sufficient to also activate PKA consistently. The evidence is incomplete and conflicting. Liver-specific CaM KKβ (CaMKK2) knockout mice expressed less hepatic G6Pase than the wild types did (Anderson et al., 2012). Although fasting for 6 h increased plasma glucagon levels into zone 2, from 18 to 87 pM, it did not alter hepatic G6Pase activity in mice (Mutel et al., 2011). However, STZ-induced diabetes of 20 weeks duration in mice increased mean plasma glucagon from 13 to 78 pM and increased the expression of G6Pase three-fold (Zhang et al., 2018). Starvation (72 h) of 2-month-old rats had no effect on plasma glucagon levels but depleted hepatic glycogen and reduced hepatic G6Pase and PEPCK activities (Bois-Joyeux et al., 1986). Neither the intracellular levels of inositol-phosphate nor those of cAMP was reported in any of these studies.

The phosphatase calcineurin is Ca2+/CaM-dependent (Chin & Means, 2000), and seems to be involved in the translocation of signal components into the nucleus. Increasing Ca2+ by activation of either pathway favors the formation of a Ca2+/CaM-calcineurin complex, increasing its phosphatase activity (Chin & Means, 2000; Hook & Means, 2001). Two important substrates of activated calcineurin are cytosolic CaMKII and CRTC2. Phosphorylation of CaMKII by activated calmodulin CaMKK enhances its activity and traps it and its substrate, CRTC2, in the cytosol (Cohen et al., 2015). Dephosphorylation of both CaMKII and CRTC2 by Ca/CaM-activated calcineurin promotes the translocation of CaMKII and CRTC2 into the nucleus. Studies of neurons suggest that, within the nucleus, Ca/CaM-activated CaMKII then activates CaMKIV, by direct interaction and via CaMKK (Cohen, 1989; Cohen et al., 2015). Activated CaMKIV then phosphorylates and activates CREB, promoting gluconeogenic gene transcription. In the liver, phosphorylation of cytosolic Fox01 by CaMKII via P38 has been proposed to promote its translocation into the nucleus to contribute to the enhancement of gluconeogenic gene transcription (Anyamaneeratch et al., 2015; Cohen et al., 2015; Erion et al., 2013; Ozcan et al., 2012).

In this context, the overlap between the two pathways in response to higher glucagon concentrations presents problems of data interpretation. For example, in mice starved for 24 h, 100,000 pM glucagon increased phosphorylation of IP3 receptors, a PKA-specific effect (Wang et al., 2012). It also increased Ca2+, an effect of activating both pathways at that high concentration. The elevated Ca2+ stimulated the CaM-dependent phosphatase calcineurin and consequent dephosphorylation of CREB-regulated transcription coactivator 2 (CRTC2), promoting its migration to the nucleus and its contribution to the enhancement gluconeogenic gene expression. Although it is very likely that both pathways were activated and involved in producing the ultimate response, the authors interpreted their results strictly within the context of the
activation of the AC/cAMP/PKA pathway alone. It would be interesting if similar results, albeit perhaps at a lower magnitude, might be achieved in response to glucagon at 60 pM, presumably in response to activation of only the PLC/IP3 pathway.

5.4 Net effects on glucose output

Under normal conditions, the contributions of glycolysis and gluconeogenesis to hepatic glucose production are approximately equal. That balance is tipped toward gluconeogenesis by fasting or starvation but variably altered by diabetes or exercise (Nordlie et al., 1999; Warner et al., 2020). The resulting transcriptional and post-translational responses to activation by glucagon of the PLC/IP3/Ca/CaM pathway at physiological concentrations—and of the AC/cAMP/PKA pathway at higher concentrations—is an elevation in either glycolysis or gluconeogenesis and in net glucose output (Table 2D). Relationships between physiological glucagon concentrations and changes in selected intracellular targets of the PLC/IP3 pathway are shown in Figure 5. Between 27.8 (10^{1.44}) and 60.0 (10^{1.78}) pM, the physiological hepatic portal glucagon concentration range illustrated in Figure 1, glucagon increased: Ca^{2+}_{i} up to approximately 50% and GPase activity to 90% (Figure 5a); F-1,6-BPase activity to 85% and its phosphorylation to 55% (Figure 5b), the expression and thus the activity of the gluconeogenic enzyme PEPCK to 70%, and gluconeogenesis to 60% of the maxima generated by higher concentrations (Figure 5c). It also inhibited the activities of PyrK and GS up to 40 and 90%, respectively (Figure 5d). The responses of these downstream effects to 60 pM correspond to a net increase in glucose output of approximately 40% of the maximum (Figure 4). These independent studies provide further evidence that glucagon, at plasma concentrations that prevail most of the time in vivo, even in type 2 diabetes, produces robust intracellular effects leading to enhanced glucose output by activating the PLC/IP3 pathway without activating the AC/cAMP pathway. This conclusion would be further supported by demonstrating substantial gaps between minimal concentrations required to generate glucose-mobilizing responses, by activating the PLC/IP3 pathway, and higher TCs required to activate the AC/cAMP pathway, measured in the same study to avoid between-laboratory variability (Table 2D, Figure 5).

5.5 Threshold concentrations of glucagon required to generate glucose-mobilizing responses and activation of hepatic AC determined in the same study

Such comparisons were made in four of the studies selected from Table 2 and listed in Table 3. The concentration gaps were revealed by comparisons of TCs of two glucagon dose-response curves: one—with a lower concentration range—for generating the indicated glucose-mobilizing response, and the other—over a higher range—for increasing either tissue cAMP levels or AC activity. As Table 3 shows, all of the minimal concentrations required to produce glucose-mobilizing responses were either 10 or 20 pM, but those necessary to produce AC-activating responses were much higher, 200–1000 pM. The ratios of TCs required to activate the AC/cAMP pathway to those sufficient to activate the PLC/IP3 pathway varied from a low of 10 to a high of 100. Overall, these findings provide additional persuasive evidence that the concerns raised by Sutherland and coworkers in 1971 as discussed above—that hepatic portal plasma concentrations may be too low

| TCs (pM) required: | A. to generate the glucose-mobilizing response | B. to increase AC activity or cAMP levels | B/A | Reference |
|------------------|---------------------------------------------|------------------------------------------|-----|-----------|
|                  |                                             |                                          |     |           |
| 20               | 200                                         | 10                                       |     | Exton, Lewis, et al. (1971) |
| 10               | 250                                         | 25                                       |     | Wakelam et al. (1986)       |
| 10               | 400                                         | 40                                       |     | Blackmore and Exton (1986)  |
| 10               | 1000                                        | 100                                      |     | Corvera et al. (1984)       |

Note: All values are from those references cited in Table 2 that reported both glucagon TCs–one to generate the indicated response and the other to activate AC or increase tissue cAMP levels—in the same study. Glucose-mobilizing responses include the stimulation of inositol-phosphate generation, GPase activity, and glycogenolysis. Note that all of the TCs sufficient to generate the responses (10 or 20 pM) are at the low end of zone 1 (physiological; 0–60 pM). By contrast, all of the TCs sufficient to activate AC or increase tissue levels of cAMP in these studies are in zone 3 (physiological hyperglucagonemia; 100–800 pM), or zone 4 (pharmacological; >800 pM) (Figure 2).
to activate hepatic AC—seem to be valid after all, at least for adult mammals under normal metabolic conditions. The findings summarized in Table 3 can be extended to hypothesize that all of the glucose-mobilizing responses listed in Table 2 were produced by activation of the PLC/IP3 signal pathway without activation of AC or PKA, because all of the TCs are at or below 40 pM (Table 3).

6 | QUESTIONS OF SIGNAL AMPLIFICATION

Observations that glucagon can stimulate hepatic glucose mobilization at physiological concentrations without measurably increasing tissue cAMP levels cannot be explained by its activation of a latent, amplified AC/cAMP pathway that is below the limit of detection.

6.1 | The AC/cAMP pathway

An argument that has been occasionally put forward is that the AC/cAMP/PKA pathway in the adult liver is indeed activated by physiological plasma concentrations of glucagon, but the signal is latent, below the limit of detection by conventional methods. Recall that the consensus TC for activating AC ex vivo is 100 pM (Figure 4), but mean hepatic portal plasma glucagon concentrations are around 44 pM (Figures 1, 2b, and 4). The question is whether the AC/cAMP pathway contributes to the mediation of glucagon’s glucose-mobilizing effects at physiological concentrations in vivo but its activation of that pathway is not detectable by conventional methodologies. A hidden role for cAMP at low glucagon concentrations had been suggested very early on by Exton and coworkers (Exton, Lewis, et al., 1971; Exton, Robison, et al., 1971): “The fact that glycogenolysis was stimulated in these experiments by concentrations of glucagon lower than those that produced a measurable elevation of tissue cyclic AMP [200 pM in their hands] suggests that the increase in nucleotide required to activate phosphorylase is extremely small, and that measurements of tissue nucleotide are a poor index of the level of metabolically active compound.” It should be borne in mind that they made that statement fifteen years prior to the publication of the discovery of the other signal (Wakeham et al., 1986). Nevertheless, because AC can be compartmentalized (Yamatani et al., 1998), PKA can be localized near the plasma membrane of the cell, cAMP phosphodiesterases can be in close proximity to the localized AC enzyme (Ong & Ambudkar, 2020; Taskén & Aandahl, 2003), and glucagon can stimulate hepatic phosphodies- terases (Heyworth & Houslay, 1983), it is theoretically possible that binding of a small fraction of the available GR2 receptors can generate enough cAMP to briefly and locally activate PKA, with amplification of the signal pathway downstream (Christophe, 1995; Jelínek et al., 1993). Responses to concentrations below 100 pM could thus be mediated by both pathways acting in concert, one overt and one latent (Figures 1, 2, 4, and 6).

Results of a key study carried out by Cherrington and coworkers (Rivera et al., 2010) seem to refute this hypothesis. The main AMPKα phosphorylation target for CaMKKβ is Thr172, and one for PKA is Ser485 (Fujiwara et al., 2016; Hurley et al., 2006; Jacquel et al., 2018; Longuet et al., 2008; Salminen et al., 2016). Infusion of glucagon into anesthetized dogs increased plasma glucagon concentration from 14 pM to a fairly steady 57 pM (Rivera et al., 2010). At those concentrations, sufficient to increase PEPCK expression and substantially enhance glucose output ex vivo (Figure 4), glucagon increased the phosphorylation of AMPKα at Thr172, the CaMKKβ phosphorylation site, but did not enhance the phosphorylation of the enzyme at Ser485, the PKA target. These results support the central thesis of this review, that the day-to-day regulation of hepatic glucose mobilization by glucagon—in healthy, fed and short-term fasted experimental animals and humans—is mediated by the PLC/IP3/Ca/CaM pathway exclusively. Additional evidence against the latent signal hypothesis may have been provided by the results of a study carried out by McKnight and coworkers (Willis et al., 2011). Substantial (app. 56%) liver-specific inhibition of PKA in mice did not diminish 24-h fasting-induced increases in the expressions of PEPCK, G6Pase (Figure 6), glyceraldehyde-3-phosphate dehydrogenase, the glucose transporter GLUT2, or glucose disposal. Growth and development were not different from those of the wild type. Plasma glucagon levels were not measured. These findings can be interpreted at least two ways: (1) The AC/cAMP pathway does not contribute to the regulation by glucagon of hepatic glucose mobilization in fasting mice at rest. Instead, the exclusive mediator is the PLC/IP3 pathway; or (2) The residual PKA activity may have been sufficient to at least contribute to the mediation of glucagon’s effects. Indirect evidence supports the first interpretation. Mice with liver-specific knockout of the Gαs subunit, eliminating activation by glucagon of GR2 receptors coupled to AC but not of GR1 receptors coupled to Gq and PLC, had normal lifespans, growth rates, and metabolic rates, increased glucose tolerance, and normal fasting glucose levels in spite of reactive hyperglucagonemia (Chen et al., 2005).
Although glucagon does not appear to activate an amplified AC/cAMP pathway, it does seem to activate an amplified PLC/IP3 signal at physiological concentrations to stimulate glucose mobilization.

### 6.2 The PLC/IP3 pathway

Although glucagon does not appear to generate an amplified AC/cAMP signal, it does seem to activate an amplified PLC/IP3 pathway at physiological concentrations. As mentioned earlier, the magnitude of the increase in IP3 in the response to physiological concentrations of glucagon is small when compared to the relatively robust increase in glucose output (Figure 4b). “Since the concentrations of IP3 produced by glucagon are significantly smaller than those produced by the ‘classical’ Ca\(^{2+}\)-mobilizing hormone vasopressin, it has been difficult to ascertain the role of a PLC-dependent mechanism in physiological effects of glucagon” (Aromataris et al., 2006). The plausibility that activation of GR1 receptors generates an amplified signal downstream, however, is supported by histological observations. As with the AC/cAMP pathway, components of the PLC/IP3 pathway are localized and compartmentalized within or close to the cell membrane. Much of the ER membrane containing the IP3Rs is closely applied to the plasma membrane (Feriod et al., 2014; Ong & Ambudkar, 2020). The proximity of GR1 receptors and PLC in the plasma membrane to IP3Rs in the ER has been proposed to facilitate signal transfer to an extent that is sufficient to produce a substantial increase in cellular calcium and related downstream events leading to enhanced glucose output (Hansen et al., 1998; Ong & Ambudkar, 2020; Williamson et al., 1987). As discussed above, concentrations of glucagon that produce no measurable increase in tissue cAMP nonetheless produce substantial increases in glucose output (Figure 4), glycogenolysis, gluconeogenesis, and altered activities of glucose-mobilizing enzymes (Table 2, Figure 5) that can only be attributed to activation of the PLC/IP3 pathway. Even though IP3 is only 10% of all inositol-phosphates generated by glucagon (Williamson et al., 1987), the “... small increase in IP3 is sufficient to fully activate phosphorylase” (Keppens et al., 1993) and presumably to produce other signal-related downstream events as well (Figure 5), leading to enhanced glucose output without activation of AC. With regard to the question of signal amplification, the major difference between the generation of IP3 and of cAMP is that, within...
the physiological, zone 1 glucagon concentration range, at or below 60 pM, the former is small but consistently measurable while the latter is undetectable because it is not generated above constitutive levels (Figure 4) (Table 2, Figures 4 and 5).

The regulation by glucagon of hepatic glucose mobilization in type 2 diabetes seems to be mediated by the PLC/IP3 pathway exclusively. Whether it is also mediated by the AC/cAMP pathway in type 1 diabetes or starvation defies prediction.

7 | UNCERTAIN INVOLVEMENT OF THE AC/cAMP PATHWAY IN HEPATIC RESPONSES TO GLUCAGON: DIABETES AND STARVATION

7.1 | Unpredictability of the AC/cAMP response to transitional concentrations of glucagon

Evidence presented above seems to establish that mean hepatic portal plasma concentrations that prevail most of the time—that is, in metabolically unchallenged experimental animals and humans at rest—are in zone 1, below 60 pM by RIA (Figures 1 and 2b). At those concentrations glucagon regulates hepatic glucose mobilization exclusively by activating the PLC/IP3 pathway without activating AC. Those plasma concentrations are consistently found in healthy or type 2 diabetic experimental animals or humans. By contrast, at zone 3 concentrations, above 100 pM, glucagon predictably activates AC (Figure 4a) so that its influences on glucose mobilization are mediated by a combination of the AC/cAMP and PLC/IP3 pathways. Those supraphysiological concentrations are characteristic of early neonates or exercising adults (Figure 2d) (Figures 1, 2, and 4).

However, within the transitional zone 2 range, between 60 and 100 pM, the effect of glucagon on AC activity is unpredictable, even ex vivo. Plasma concentrations in starving or T1D experimental animals or humans can approach or be within that range (Figure 2c). There is an apparent gap between results obtained ex vivo, which seem to show that AC is activated minimally or not at all (Figure 4), and those generated in vivo in these conditions, indicating that glucagon can inconsistently and substantially activate AC and increase tissue cAMP levels even below these transitional plasma concentrations. With regard to ex-vivo variability, recall that in the concentration-effect curve in Figure 4, the glucagon TC of 100 pM increased cAMP levels—in perfused livers, hepatocytes, or hepatocyte membranes—by a composite mean of only about 4% of the maximum response to higher concentrations, but the individual responses were somewhat variable. Of the 15 references used to calculate that 4% mean, 6 (including the original communication by Sutherland and coworkers) reported no effect (Corvera & García-Sáinz, 1984; Exton, Robison, et al., 1971; Lynch et al., 1989; Robberecht et al., 1988; Soman & Felig, 1978; Unson et al., 1989). The other 9 (Clark & Jarrett, 1978; Dich & Guluud, 1976; Dighe et al., 1984; England et al., 1983; Hermsdorf et al., 1989; Pohl et al., 1972; Rodbell et al., 1971; Sonne et al., 1978; Yagami, 1995) reported increases in either tissue cAMP levels or AC activity between 2% and 21% of the maximum. According to at least 3 additional sources, transitional concentrations are indeed capable of significantly activating AC either ex vivo or in vivo. Glucagon at 100 pM was reported to increase cAMP levels 86%, PKA activity 62%, and glucose output 90% over basal levels in hepatocytes isolated from fed rats (Assimacopoulos-Jeannet et al., 1977). In perfused rat livers, 60 pM glucagon increased glucose output by about 87% of the maximum produced by 1000 pM, and increased tissue cyclic AMP 3-fold, from 0.5 to 1.5 pmol/g (Doi et al., 2001). Co-infusion with insulin completely blocked the increase in cAMP but reduced the peak increase in glucose output by only 25%. The residual response was presumably the result of activation of the PLC/IP3 pathway. The authors apparently did not specify whether the livers were isolated from fed or fasted rats. One report is unusual with regard to the magnitude of the increase in cAMP in this concentration range (Perry et al., 2020). At the end of a 2-h infusion of glucagon into overnight-fasted mice, plasma glucagon levels had increased from 9 pM into zone 2, approximately 80 pM, and hepatic cAMP had risen from 300 to 1650 pmoles/g, an increase of over 5-fold. Values at earlier time points were not reported. As mentioned above, transitional glucagon concentrations can be achieved in at least two metabolically stressful conditions, insulin-dependent diabetes (TID) and starvation (Figure 2c). Not surprisingly, attendant effects on hepatic cAMP or AC activity in those conditions are variable.

7.2 | Diabetes

It is becoming increasingly clear that hepatic glucose-mobilizing effects of glucagon likely contribute to the hyperglycemia and related complications of diabetes (Foretz et al., 2005; Lee et al., 2012; Müller et al., 1973; Patil et al.,
Whether the elevation in blood glucose is associated with a rise in plasma glucagon seems to depend on the category of diabetes. The “hyperglucagonemia” of diabetes (Rix et al., 2019) appears to be nonexistent in either obesity with insulin resistance or T2D (hereafter referred to collectively as T2D) (Figure 2c), but it may be present at moderate levels in insulin-dependent T1D (Figure 2c, Table 4A). As expected, hepatic AC/cAMP levels in T2D are basal and characteristic of those in nondiabetic animals, but those in T1D are variable and may exceed nondiabetic levels (Table 4A). This difference can be attributed, at least partially, to the disparity of mean plasma glucagon concentrations; normal in T2D but variably elevated in T1D. Respective effects of altered insulin action or availability may also be involved (Tables 4 and 5, Figures 2, 4, and 7).

Declines in either plasma insulin or the sensitivity to insulin in diabetes would be expected to lift its inhibition of cAMP-mediated responses to glucagon (Gabbay & Lardy, 1984; Illiano & Cuatrecasas, 1972). Sites and mechanisms of inhibitory actions of insulin include suppressed glucagon secretion from alpha cells (Ravier & Rutter, 2005) and, in the liver, reduced basal and glucagon-stimulated AC/ PKA activities (Claus et al., 1979; Heyworth & Houslay, 1983a; Seitz et al., 1977) and accelerated cAMP hydrolysis by stimulation of cAMP-dependent phosphodiesterase (Heyworth et al., 1983b; House et al., 1972; Houslay, 1990; Loten et al., 1978). Treatment with anti-insulin antibodies in vivo increased basal cAMP of perfused livers ex vivo (Jefferson et al., 1968). Opposing nuclear effects of insulin include inhibition of the expressions or activities of PGC-1α, CREB, and PEPCK (Christ et al., 1990; Hatting et al., 2018; Herzig et al., 2001). In theory, these nuclear anti-glucagon effects of insulin would apply to either of the glucagon-activated signal pathways. However, insulin resistance in T2D and its deficiency in T1D seem to have differing effects on plasma glucagon levels and their relationships to hepatic AC/cAMP activity.

### Table 4

| Condition | Change in Plasma [glucagon] | Reference |
|-----------|----------------------------|-----------|
| A. Diabetic (vs. ND) | | |
| Humans, Obese IR | X | ↔ | Livingston et al. (1985) |
| Rats, T2D model | X | ↔ | Xue, Cei, et al. (2017) |
| Rats, T1D | X | ↑ | Soman and Felig (1978) |
| | X | ↓ | Walsh & Dunbar (1984) |
| | X | ↔ | Yamashita et al. (1980) |
| B. Starvation (vs. Fed) | | |
| Rats | X | ↑ | Seitz et al. (1976) |
| | X | ↓ | Srikant et al., 1977) |
| | X | ↔ | Goldstein et al. (1978) |
| | X | ↑ | Bois-Joyeux et al. (1986) |
| C. Neonates (vs. Adults) | | |
| Rats | X | ↑ | Beaudry et al. (1977) |
| D. Exercise (vs. Rest) | | |
| Rats | X | ↑ | Sellers et al. (1988), Winder et al. (1981), Winder, Yang, et al. (1988) and Winder, Arogysami, et al. (1988) |
| Dogs | X | ↑ | Issekutz (1981) |

Note: Listed are effects of the indicated condition on plasma glucagon and hepatic AC/cAMP levels determined in the same study. Experimental preparations were hepatocyte membranes (basal AC activity), hepatocytes, liver slices, or perfused livers (basal AC activity or tissue cAMP levels). In STZ-induced diabetes or starvation (A and B), both plasma glucagon and associated AC activities or cAMP levels are variable (see Figures 7 and 8). By contrast, in the early neonates and exercising adults, plasma glucagon levels are consistently in zone 3 and AC/cAMP levels are predictably and consistently elevated. Mean plasma glucagon concentrations (pM) in the control groups were: nondiabetic (A), 16, 8, 18, 33, 28, and 54; fed (B), 17, 28, 38, and 46; adults (C), 36; and pre-exercise (D), 52, 49, 66, 75, and 69. The composite mean for all control groups is 39.6 ± 5.1 pM (n = 16). The doses of STZ were 60–70 mg/kg, the durations of diabetes were 5–14 days, and the durations of starvation were 24–72 h.
In T2D, both plasma glucagon concentrations and basal hepatic AC/cAMP levels are within the normal range. Mean plasma glucagon levels in nondiabetic and T2D humans are similar and well within zone 1 (Figure 2a and c). According to the sources cited here, the mean peripheral venous plasma glucagon concentrations in nondiabetic and T2D humans are statistically identical, 29 and 28 pm respectively (Figure 2a and c), well within zone 1. Measured by ELISA they are even lower (Table 1). Mean plasma concentrations in normal subjects and T2D patients, measured by ELISA, were 6.0 and 9.5 pm, respectively (Kobayashi et al., 2020). Livingston et al. (1985) reported that basal, glucagon-activated, and NaF-stimulated AC activities in membrane preparations from liver biopsies taken from obese patients were not significantly different from those of lean patients. In both groups, plasma glucagon concentrations were below 30 pm. In a genetic model of T2D in the rat, the increase in hepatic cAMP produced by glucagon infusions in situ was actually blunted compared to the responses of nondiabetic controls (Doi et al., 2001). Similarly, glucagon-AC dose-response curves generated in hepatocytes or hepatocyte membranes from T2D model rats (low dose of STZ given to neonates) were depressed relative to those of the nondiabetic controls. Constitutive hepatic cAMP levels were lower in the diabetics, but glucagon receptor binding affinities and capacities and cAMP-phosphodiesterase activities were normal (Portha et al., 1983). In a diet-induced insulin-resistant rat model (Xue, Wei, et al., 2017), the animals were hyperglycemic and displayed elevated expressions of PEPCK and G6Pase but normal plasma glucagon levels and basal hepatic tissue cAMP levels. Expressions of Gsα and beta adrenceptors were elevated, but glucagon receptor expressions or densities were not reported. Evidently, in most studies of T2D plasma glucagon concentrations are normal and hepatic AC/cAMP is basal, and effects of glucagon on AC/cAMP are either normal or inhibited. It can thus be plausibly hypothesized that, because concentrations of circulating glucagon in T2D remain within zone 1 (Figure 2c), glucagon’s contribution to elevations in hepatic glucose output, and thus to the hyperglycemia, in T2D would be mediated exclusively by the PLC/IP3 pathway in vivo. But more extensive investigations will be required to confirm that hypothesis.

By contrast, the relationship between plasma glucagon concentration, hepatic glucose output, and the AC/cAMP pathway in T1D is much less clear. The contribution to the hyperglycemia attributable to glucagon-induced increases in hepatic glucose output may be mediated by GR1 activation alone or by the activation of both GR1 and GR2 receptors, depending at least in part on the magnitude of the increase in plasma glucagon concentrations. In experimental STZ- or alloxan-induced T1D, mean plasma glucagon concentrations may be normal (remain in zone 1), but may occasionally enter zone 2; that is, T1D animals may be normo- or moderately hyper-glucagonemic (Table 4A). According to the sources cited in Figure 2 for T1D, peripheral venous plasma glucagon concentrations in STZ- or alloxan-induced diabetes in rodents vary between a low of 20 pm and a high of 246 pm, with a mean inside the transitional zone, 78.2 ± 17.3 pm (Figure 2c). Based on the hepatic portal/peripheral venous concentration ratio of 1.5, as discussed above, the mean hepatic portal concentration in T1D, measured by RIA, would be over 100 pm.
However, regardless of whether the plasma concentrations in T1D are in zones 1, 2, or 3, hepatic AC/cAMP levels can be elevated, reduced, or normal, with no apparent relationship to plasma concentrations (Table 4A).

Results of ex vivo studies are also inconsistent. Prior imposition of T1D in vivo can increase, suppress, or have no effect on glucagon-stimulated AC activity in hepatic preparations ex vivo (Tables 4A and 5, Figure 7). Plasma glucagon levels and hepatic AC/cAMP activities may vary together or in different directions. Insulin-dependent diabetes can produce either a moderate increase in plasma glucagon levels (i.e., fail to increase them out of zone 1) or increase them into zone 2, yet at the same time either increase or have no effect on basal AC/cAMP. It can also increase plasma glucagon all the way into to zone 3 and actually decrease basal AC/cAMP (Table 4A). In three conflicting studies (Figure 7), T1D decreased basal AC activity and the AC-activating responsiveness to increasing concentrations of glucagon (Dighe et al., 1984), had no effect on basal activity but increased the responsiveness to glucagon (Lynch et al., 1989), or decreased basal activity but had no effect on responsiveness to glucagon (Srikant et al., 1977). In the first two studies (Dighe et al., 1984; Lynch et al., 1985), the effects of diabetes on...
plasma glucagon levels were not reported. But in the third (Srikant et al., 1977), the suppressed basal AC activity was observed ex vivo after diabetes had produced a marked increase in plasma glucagon levels in vivo (103 pM from 28 pM). Reduced basal AC activities were associated with lower densities and affinities of the GR2 receptor (Dighe et al., 1984; Srikant et al., 1977). But basal AC activities and receptor densities are not always predictive of subsequent responsiveness to glucagon. The responses may be suppressed (Dighe et al., 1984) or unaffected (Srikant et al., 1977). Furthermore, when STZ-induced diabetes does alter the position of the glucagon-AC/cAMP dose response curve in hepatocyte preparations—up or down—it displaces it vertically (i.e., it alters glucagon’s effectiveness) and not horizontally (i.e., it does not alter its potency) (Figure 7) (but see Walsh & Dunbar, 1984). Thus, the TC ex vivo for activating (or inhibiting) AC in diabetes seems to be at least 100 pM, as it is in hepatocyte preparations isolated from nondiabetic animals. By extension, when T1D does not increase plasma glucagon above 60 pM, in vivo it should not alter hepatic cAMP levels either. Yet apparently it can (Soman & Felig, 1978 in Table 4A). None of these inconsistencies is obviously related to the sex of the animals, dose of STZ or alloxan, or duration of diabetes (Dighe et al., 1984; Srikant et al., 1977; Walsh & Dunbar, 1984).

Studies are also conflicting with regard to the contribution of glucagon-induced stimulation of hepatic glucose output to the hyperglycemia of T1D. Treatment with STZ, sufficient to produce profound hyperglycemia in wild-type mice, had no effect on glycemia in glucagon receptor knockout mice (Lee et al., 2012). Results of control studies led the authors to conclude that elimination of hepatic glucose-mobilizing actions of glucagon also eliminates diabetic hyperglycemia, and that hyperglycemic actions of glucagon are more important than those of insulin withdrawal or sub-sensitivity in the pathogenesis of the disease. They did not discuss, however, whether the ameliorative effects of glucagon receptor knockout in their hands were the result of elimination of GR2 receptors coupled to AC alone or of both GR2 and GR1 receptors coupled to PLC.

In apparent conflict with those findings, administration of the glucagon-like peptide-1 (GLP-1) agonist (and inhibitor of glucagon secretion) liraglutide to STZ-diabetic rats normalized the increase in plasma glucagon without significantly affecting the hyperglycemia (Meek et al., 2015). The authors interpreted those results as evidence that the hyperglycemia of T1D is not dependent on the rise in plasma glucagon. But the increase in plasma glucagon in the diabetic animals that they observed was moderate, 21.5 pM in T1D vs. 14.4 pM in the nondiabetic controls, sufficient to increase glucose output only to 10% of the maximum according to the dose-response curve in Figure 4, and in any case well within zone 1. Thus, the stimulated output was presumably mediated exclusively by increased GR1 receptor activation. Anti-glucagon antibodies bound to and neutralized all of the circulating glucagon and consequently decreased blood glucose in nondiabetic controls by about 33% in nondiabetic controls and 40% in alloxandiabetic, hyperglycemic rabbits (Brand et al., 1996). Thus, neither the relative contribution of glucagon-induced hepatic glucose output, nor those of GR-1 and GR-1 receptor

**FIGURE 8** Variable effects of starvation on plasma glucagon and hepatic tissue cAMP levels or AC activity in rats. Depending on the study (see Table 4B), starvation was reported to increase both plasma glucagon and tissue cAMP levels (a), increase plasma glucagon but decrease hepatic AC activity (b), or have no effect on either plasma glucagon or tissue cAMP levels (c). The peak plasma glucagon levels in A and B are 56 and 57 pM, within zone 1 (Figure 2). The durations of starvation were 2 days (a), 6 days (b) and 3 days (c). The results highlight the uncertainty regarding the effect of starvation on the relationship between plasma glucagon and hepatic tissue cAMP levels or AC activity. Adapted from Seitz et al. (1976) (a), Srikant et al. (1977) (b), and Goldstein et al. (1978b) (c).
activation, to the hyperglycemia of T1D is clearly defined. Apparently, potential hypoglycemic actions of the PLC inhibitor U73122, in the absence or presence of T1D, have not been investigated. One reason for the presumed hesitancy could be that the results would be difficult to interpret because U73122 has nonspecific effects in vivo (Bill & Vines, 2020) including interference with insulin-induced increases in skeletal muscle glucose uptake (Wright et al., 2002).

To summarize, in T2D plasma glucagon levels are in the normal range, and thus would not be expected to activate hepatic AC in vivo. Any glucagon-induced increases in hepatic glucose output in that condition would presumably be mediated by the PLC/IP3 pathway exclusively. In T1D plasma glucagon concentrations are quite variable, ranging between normal and hyperglycagonecnic. Further, there is no obvious correlation between plasma concentrations and the activation of hepatic AC. Thus, the role of the AC/cAMP pathway as a co-mediator, with the PLC/IP3 pathway, of the hepatic glucose-mobilizing and hyperglycemic actions of glucagon in T1D remains unclear.

7.3 | Starvation

Starvation (defined here as fasting for 24 h or more) has complex direct and indirect effects on plasma glucagon, glycemia, and hepatic glucose metabolism. Depending on the duration, plasma glucagon levels may be fairly well maintained or may fall somewhat as starvation progresses (Blommaart et al., 1995; Mlekusch et al., 1981; Smadja et al., 1990), and plasma corticosterone concentrations can remain fairly steady or increase (Mlekusch et al., 1981; Ogias et al., 2010). Expressions of PEPCK and G6Pase rise (Krone et al., 1976; McLennell et al., 1982; Mlekusch et al., 1981; Ogias et al., 2010; Seitz et al., 1977), while plasma insulin-to-glucagon ratios generally decline (Aguilar-Parada et al., 1969; Bois-Joyeux et al., 1986; Goldstein et al., 1978; Mlekusch et al., 1981; Shiota et al., 1996; Smadja et al., 1990; Verrillo et al., 1988). Increases in PEPCK expression and gluconeogenesis during starvation are mediated in part by declines in hepatic levels of G protein receptor-coupled kinase 2 (GRK2) (Cruces-Sande Arcones et al., 2020). Decreases in GRK2 inhibit the internalization of G proteins associated with glucagon receptors (and other GPCRs), preserving their responses to starvation-induced increases in plasma glucagon concentrations (when they occur) (Table 4, Figures 2, 6, and 8).

As in experimental T1D, in starvation the relationship between plasma glucagon and hepatic AC/cAMP is variable and difficult to predict. According to the references cited here (Figure 2c), the collective mean peripheral venous plasma glucagon concentration in starvation of rats or humans is 48 pM, approaching zone 2. All of the measurements used to generate that mean were taken by RIA from the peripheral venous circulation. Applying the hepatic portal/peripheral venous ratio of 1.5 (Figure 2b), the corrected concentration in the hepatic portal circulation would be 72 pM, well into the transitional zone 2. The mean values, measured in the peripheral venous circulation, varied from a low of 34 to a high of 66 pM. Even when corrected, none enters zone 3 (exceeds 100 pM) and thus would not be expected to increase hepatic AC activity or cAMP levels. However, hepatic AC activity or cAMP levels may increase anyway (Seitz et al., 1976), but they may also decrease (Srikant et al., 1977), or remain unaltered (Goldstein et al., 1978), with no obvious relationship to attendant changes in plasma glucagon levels (Table 4B, Figure 8). In one representative report, starving for 24 h slightly decreased plasma glucagon in rats from 57 ± 4 to 50 ± 11 pM, but increased hepatic tissue cAMP by over 60%, from 0.16 ± 0.01 to 0.26 ± 0.02 nmol/g wet wt. (Goldstein and Curnow (1978)). After starving for 120 h, plasma glucagon concentrations rose to 107 pM, and hepatic cAMP levels further increased to 0.40 nmol/g. In contrast, rats starved for 6 days exhibited increased plasma glucagon from 28 to 57 pM, but reduced hepatic AC activity from 1.27 to 0.64 nmol/10 min · mg prot.−1 (Srikant et al., 1977). As mentioned above, Willis et al. (2011) reported that liver-specific inhibition of PKA (by about 60%) in adult mice had little or no effect on hepatic responses to starving for 24 h (Figure 6). It did not suppress starvation-induced increases in hepatic PEPCK or G6Pase expression or changes in blood glucose levels. In the fed and starved states, both PKA-inhibited and wild-type mice had similar or identical expressions of hepatic glucokinase, G6Pase, and GLUT-2 transporters, and increases in the starvation-induced expression of GAPDH, hepatic glucose disposal, body weights, liver weights, and blood insulin levels. As discussed above, the results can be interpreted at least two ways: (1) The effects of starvation do not involve activation of the AC/cAMP pathway; or (2) The residual basal PKA activity was sufficient to mediate or at least contribute to the responses. The authors did not measure plasma glucagon levels, but it seems likely that they were within zone 1, below 60 pM, in the fed controls (Figures 1 and 2a). Interestingly, PKA inhibition did significantly suppress expressions of PEPCK and G6Pase in that group. A possible implication of those findings is that the expressions of PEPCK and G6Pase are maintained at minimal levels at least in part by a steady, constitutive, unstimulated AC/cAMP/PKA signal at plasma glucagon levels below at least 60 pM. Expressions of these enzymes above the basal level would then be controlled by variations in plasma glucagon concentrations within that range, mediated by
the PLC/IP3/PLC pathway exclusively. To carry that speculation to an extreme, this principle might apply to all intracellular targets—shared by the two pathways—that are involved in the regulation of hepatic glucose mobilization. In starvation, therefore, as in T1D, neither the relationship between plasma glucagon concentrations and hepatic tissue cAMP levels nor the role of the AC/cAMP signal pathway in mediating glucagon’s glucose-mobilizing effects is clearly established. Glucagon’s control of hepatic glucose mobilization in these conditions may be mediated by the PLC/IP3 pathway alone or in combination with the AC/cAMP pathway.

In contrast to its variable effects in type 1 diabetes and starvation, in early neonates or exercising adults, glucagon consistently activates the hepatic AC/cAMP pathway. Thus, the hormone adaptively boosts hepatic glucose output in order to meet the elevated systemic demand for glucose in both of these metabolically stressful conditions.

8 ROLE OF THE AC/cAMP PATHWAY IN MEETING ELEVATED GLUCOSE DEMAND IN EARLY NEONATES AND EXERCISING ADULTS

As discussed above, when systemic glucose supply/demand ratios fall to critical levels, plasma glucagon concentrations consistently rise from zone 1 into zone 3 (physiological hyperglucagonemia) and thus predictably activate both the AC/cAMP and PLC/IP3 pathways simultaneously. As the ex vivo data presented in Figure 4 predict, at those higher concentrations activation of the AC/cAMP pathway serves as a backup signal in vivo, supplementing a PLC/IP3 pathway that is also further stimulated, boosting hepatic glucose mobilization to meet the elevated systemic glucose demand. This pattern is consistently found in two metabolically stressful conditions, the early neonate and the exercising adult.

8.1 Early neonates

Neonates are at high risk of hypoglycemia, and are uniquely dependent on the nutritional provision of exogenous glucose or, failing that, of gluconeogenic substrates, to maintain glycemia (Decaux et al., 1986; Girard, 1986; Girard et al., 1973; Mehta et al., 1987; Stanley et al., 2015). In the early neonate, moderate hyperglycemia minimizes the risk of hypoglycemic damage, particularly to the developing central nervous system (Gièmes et al., 2016; Hume et al., 2002). Whole-body knockout of cytosolic PEPCK, for example, is lethal in the early postnatal period (Semakova et al., 2017). Premature-born infants appear to have abnormally low peak plasma glucagon levels, a blunted glucagon response to variable nutritional conditions (Bak et al., 2014; Hawdon et al., 1993; Hawdon et al., 1993; Hume et al., 2002; Jackson et al., 1997; Mehta et al., 1987; Molinari et al., 1982; Sunehag et al., 1994), and suppressed responses to exogenous glucagon (Hume et al., 2002). This helps to explain why premature infants are more vulnerable to damaging effects of fasting or restricted nutrient availability than are full-term neonates (Table 4C, Figures 2d and 9a).

Metabolic and hormonal changes begin prior to birth in anticipation of the elevated glucose demand in the early neonatal period. In utero, glucose availability is sufficient in the maternal/fetal circulation, as long as maternal glycemia is not compromised. Prior to the abrupt transition from maternal to autonomous circulation at birth, hepatic glycogen stores begin to increase (Mayor & Cuezva, 1985). Relative alpha cell mass (μg/g BW) also begins to expand in utero, and can reach levels in 4-day-old neonates nearly 20 times greater than that of the adult (Fernández-Milán et al., 2013; Movassat et al., 1997). In rats and humans, plasma glucagon spikes within the first hours after birth (Aalinkeel et al., 1999; Fernández-Milán et al., 2013; Hahn et al., 1977; Lyonnet et al., 1988; Ogata et al., 1987, 1988), with mean levels that can exceed 500 pM (Girard, 1990; Luyckx et al., 1972) (Figure 2d). Hepatic gluconeogenesis does not become active until shortly after birth (Schaub et al., 1972) and increases substantially and rapidly to levels above those in the adult liver (Beaudry et al., 1977). The high insulin-to-glucagon ratio in utero is quickly and substantially reversed in the early neonate (Fernández-Milán et al., 2013; Girard, 1990; Hahn & Hassanali, 1982; Ktorza et al., 1985; Lyonnet et al., 1988). A reduced counterregulatory influence of insulin on hepatic glucose handling, potentially affecting either glucagon-activated pathway, is an important hormonal component of the adaptive response in the neonate (Girard, 1990; Hahn & Hassanali, 1982; Mlekusch et al., 1981; Nurjan et al., 1985). A rise in plasma corticosteroid levels contributes to the enhancement of gluconeogenic gene expression (Ogias et al., 2010). Further, the rate of hepatic glucagon degradation is much lower in the fetus and early neonate than it is in the adult (Vinicor et al., 1976), presumably increasing local concentrations in the hepatic circulation and prolonging and intensifying the glucose-mobilizing effect.

A supplementary capacity for hepatic glucose mobilization contributes to meeting the higher systemic glucose
demand in the neonate compared with that in the adult (Hahn et al., 1977; Hahn & Hassanali, 1982; Ktorza et al., 1985; Mayor & Cuezva, 1985). Glycogen stores, built up in utero, are rapidly depleted postnatally, particularly during periods of fasting (Biondi & Viola-Magni, 1977; Nurjhan et al., 1985). Compared to measurements taken from the adult, in the neonate plasma glucagon, corticosterone, and catecholamines are elevated and insulin levels much lower, while the activities and expressions of PEPCK, G6Pase, PK, and F-1,6-DP are all elevated (Baly et al., 1985; Beaudry et al., 1977; Biondi & Viola-Magni, 1977; Girard, 1986, 1990; Lyonnet et al., 1988; Mayor & Cuezva, 1985; Noguchi et al., 1985; Ogata et al., 1987, 1988; Slotkin et al., 1995). Starvation in 3-week-old neonates for 24 h further increases plasma glucagon and PEPCK activity, depletes hepatic glycogen, and decreases plasma glucose and plasma insulin-to-glucagon ratios (Claeyssens et al., 1992).

Predictably, the hepatic AC/cAMP pathway is more active and responsive to glucagon in the neonate than it is in the unstressed adult. Both basal and glucagon-activated hepatic AC activity (Beaudry et al., 1977; Girard, 1990; Slotkin et al., 1996; Vinicor et al., 1976) are markedly elevated in the neonatal liver compared to those of the adult (Table 4C). These findings are consistent with the observations that glucagon receptor densities (Pingoud et al., 1982) and the Km and Vmax of AC are higher than they are in the adult (Vinicor et al., 1976). As the animal ages
and chronic systemic demand for glucose progressively declines in the fed or short-term fasting state, relative alpha cell mass, plasma glucagon, insulin, and insulin-to-glucagon ratios, corticosteroid and catecholamine concentrations, hepatic constitutive AC activity and cAMP levels, and basal rates of glucose output, steadily approach adult levels. The evidence therefore strongly supports the view that, in the early neonatal period, physiological hyperglucagonemia (Figure 2d) strongly and consistently activates the AC/cAMP pathway, enhancing activities and expressions of gluconeogenic enzymes and hepatic glucose output above adult levels in order to meet the elevated systemic glucose demand. The relative contribution of the PLC/IP3 pathway to the regulation of glucose mobilization in the neonatal liver has apparently not been investigated. It would likely be strongly activated, based on the glucagon-inositol-phosphate curve for adults shown in Figure 4.

In contrast with the findings discussed above, it has been reported that the activation of the AC/cAMP pathway by glucagon is paradoxically depressed in the neonate (Beaudry et al., 1977). But that interpretation may depend on how the data are presented. When expressed in relative terms (% of basal levels that are normalized to 100%) as originally reported, glucagon-stimulated cAMP levels appear to be lower in neonatal rat hepatocytes than they are in adult hepatocytes (Figure 9a). However, when the same data are instead converted to absolute tissue concentrations (pmoles/mg), as they have been here (Figure 9b), both basal and glucagon-stimulated cAMP levels are revealed to be higher in neonatal hepatocytes than they are in adult cells, consistent with the findings discussed above. Predictably, the elevated basal cAMP levels in the hepatocytes were evident over the glucagon concentration range corresponding to physiological hepatic portal plasma levels shown in Figure 1, while the enhanced glucagon-simulated cAMP levels were observed at higher concentrations representative of those in neonatal plasma. The relative response to higher glucagon concentrations is lower because the basal levels are higher. It should be acknowledged, however, that neither the higher basal cAMP levels nor the increased AC/cAMP responsiveness to glucagon in the neonate, even when expressed in absolute terms, is consistently reported (Pingoud et al., 1982; Vinicor et al., 1976). The disparate results remain to be resolved.

8.2 Exercising adults

As in the early neonate, in adult mammals undergoing strenuous exercise metabolic demand for glucose is elevated. Acute hormonal responses to exercise recapitulate the neonatal situation at least four ways: higher plasma glucagon; increased hepatic cAMP levels; elevated plasma corticosteroid levels; and diminished plasma insulin-to-glucagon molar ratios (Banzet et al., 2009; Watanabe et al., 1991; Winder, 1988; Winder et al., 1988). As they do in the neonate, all four work in concert to increase hepatic glucose output in the exercising adult (Table 4D and Figure 2d).

Strenuous exercise, especially when combined with fasting or starvation, seems to be a powerful stimulus for recruitment of the AC/cAMP pathway to adaptively boost hepatic glucose mobilization (Table 4D). Exercise, alone or in combination with starvation (Winder et al., 1979, 1982), can increase mean plasma glucagon concentrations into zones 2 or 3. According to the 9 sources listed in Figure 2d, the collective mean is 236 ± 83 pM with a range of 32 to 717 pM. When plasma glucagon levels in exercise rise above 100 pM, the AC/cAMP pathway is consistently activated (Table 4D). This is associated, in both exercise and fasting, with increased hepatocyte glucagon receptor densities, but with increased receptor affinities only in response to fasting (Mehta et al., 1987; Melanç). After 20 min of exercise, both fed and overnight-fasted rats displayed depleted hepatic glycogen levels (Winder et al., 1979). Exercise of rats to exhaustion increased the hepatic expression of PEPCK 4-fold (Banzet et al., 2009). Strenuous exercise increased plasma glucagon from 66 to 124 pM, reduced plasma insulin from 290 to 90 pM, and increased hepatic tissue cAMP levels from 0.73 to 1.36 pmoles/mg (Winder, Yang, et al., 1988). Rats subjected to a 24-h period of starvation and an uphill treadmill exercise for 60 min displayed a four-fold rise in plasma glucagon (from 40 to 167 pM), a 50% drop in plasma insulin, an increase in liver cAMP of 54%, a decline in blood glucose of 55%, a ten-fold increase in plasma corticosteroids, and nearly 100% depletion of liver glycogen (Winder et al., 1982). After 20 min of exercise, plasma glucagon was elevated and hepatic glycogen was depleted in both fed and overnight-fasted rats, but hepatic cAMP was elevated only in the fasted animals (Winder et al., 1979). In retrospect, the cAMP-independent action of glucagon to deplete hepatic glycogen in the exercising fasted animals can be explained by activation of the PLC/IP3 pathway alone.

In summary, the metabolic adjustments in the exercising adult recapitulate those in the early neonate in at least two important ways: (1) Plasma glucagon levels markedly increase; and (2) The higher levels of glucagon consistently activate the AC/cAMP pathway to boost hepatic glucose output in order to adaptively meet the elevated systemic glucose demand. In this regard, the role of the AC/cAMP pathway in mediating the effects of glucagon is much clearer than it is in T1D or starvation alone.
Glucocorticoids may inconsistently enhance the ability of glucagon to increase hepatic tissue levels of cAMP by increasing glucagon’s effectiveness but not its potency in activating AC.

9 | POSSIBLE ROLE OF ELEVATED GLUCOCORTICOIDS IN STARVATION AND DIABETES

Plasma corticosteroids are elevated in early neonates, exercising adults, starvation, and T1D (Huang et al., 2006; Ogias et al., 2010; Schwartz et al., 1997; Watanabe et al., 1991). In the first two conditions, any influence of elevated corticosteroid levels on the extent of activation of hepatic AC/cAMP by glucagon is obscured by the marked increase in plasma glucagon levels (Figure 2d), well above 100 pM and thus sufficient to consistently and robustly activate AC on its own (Table 4C and D). In starvation and T1D, however, plasma glucagon concentrations do not rise as high; they can either stay in zone 1 (below 60 pM) or increase into the transitional zone 2 (60–100 pM), but very rarely go above 100 pM. Not surprisingly, the influence of glucagon on hepatic AC/cAMP in these conditions is inconsistent, as discussed above. One possible explanation for the inconsistency is that attendant increases in plasma corticosteroid levels have variable effects on the activation of the AC/cAMP pathway and stimulation of glucose output by glucagon at transitional concentrations (Figure 10).

Corticosteroids can either act on their own or interact with glucagon to influence hepatic gluconeogenesis. Glucocorticoids, by interacting with the glucocorticoid receptor and the glucocorticoid response element, activate a number of transcription factors involved in the expressions of PEPCK and G6Pase, ultimately stimulating gluconeogenesis (Jitrapakdee, 2012). They synergize with cAMP to induce the transcriptional activator PGC-1 and gluconeogenesis (Banzet et al., 2009); PGC-1 increases expressions of both PEPCK and G6Pase (Yoon et al., 2001). In addition, corticosteroids are often described as having a “permissive” effect on the generation of cAMP by high concentrations of glucagon and by other AC activators such as NaF, isoproterenol, or forskolin (Adigun et al., 2010; Exton, 1979; Exton et al., 1972; Krone et al., 1976; Yoon et al., 2001). The nonselective action has been termed “heterologous sensitization” (Adigun et al., 2010). Liver-specific knockout of the glucocorticoid receptor suppressed the enhanced expression of PEPCK in both STZ-diabetic and 48-h starving normoglycemic mice, and blunted the hyperglycemia in the former and exacerbated the hypoglycemia in the latter (Opferk et al., 2004). Enhancement of glucagon’s AC/cAMP-mediated effects may also involve glucocorticoid-induced inhibition of cAMP phosphodiesterase (Manganiello & Vaughn, 1972) and increased expressions of Gas1 and Gas2 in both neonates and adults (Kawai & Arinze, 1993).

The permissive or sensitizing effect of corticosteroids on responses to AC-activating concentrations of glucagon is observed ex vivo but not consistently in vivo. Administration of dexamethasone to cultured rat hepatocytes enhanced the effectiveness of glucagon to increase cAMP levels by displacing the concentration-effect curve upward (Figure 10a), but without altering its potency (i.e., it did not affect the TC, which in this case was 630 pM). These results suggest that elevations in plasma glucocorticoids would enhance the AC-activating actions of glucagon in vivo only if plasma glucagon concentrations rise above 100 pM. Consistent with that view, administration of dexamethasone (10 or 50 μg/kg/d, 26d) to metabolically unstressed rats, whose plasma glucagon levels were presumably below 60 pM, had little to no effect on AC activity in rat liver (Slotkin et al., 1996).

It follows that the question of whether the ability of glucagon to inconsistently activate the AC/cAMP pathway at concentrations above 60 pM, in T1D or starvation, can be attributed at least in part to permissive effects of corticosteroids has not been answered definitively. As discussed above, the relationship between plasma glucagon and hepatic AC/cAMP in T1D or starvation is variable (Figures 7 and 8), and not always correlated with changes in plasma corticosteroids. Withdrawal of endogenous corticosteroids by adrenalectomy might be predicted to suppress the concentration-dependent stimulation of the AC/cAMP pathway by glucagon ex vivo. But that is not borne out by the results shown in Figure 10b; adrenalectomy had no effect on glucagon’s activation of AC or PKA in hepatocytes (Chan et al., 1979; Seitz et al., 1976). As a control (Figure 10c), adrenalectomy did inhibit basal GPase activity and its enhancement in response to glucagon at concentrations between 100 and 7000 pM. It also suppressed the glucagon concentration-effect curve for glucose output (Chan et al., 1979). In contrast, adrenalectomy slightly increased exercise-induced elevations in hepatic cAMP levels without altering plasma glucagon levels (Sell et al., 1988). Starvation of rats for 48 h increased serum glucagon (from about 25 to 55 pM), hepatic PEPCK, and tissue cAMP to the same extent in control and adrenalectomized rats, while not markedly affecting serum insulin levels in either group (Seitz et al., 1976).

Glucocorticoids may also influence responses to glucagon that are mediated by the PLC/IP3 pathway, but the evidence is indirect. Adrenalectomy suppressed the stimulation of glucose output or gluconeogenesis in perfused
FIGURE 10 Effects of acute dexamethasone administration ex vivo (a) or prior adrenalectomy (b and c) on glucagon concentration-dependent increases in cAMP generation (a, b), or phosphorylase a (GPase) activity (c) in rat hepatocytes. Administration of dexamethasone “sensitized” the hepatocytes by increasing the maximal response, but did not alter the TC of 630 (102.80) pM (a). Adrenalectomy in vivo had no effect ex vivo on glucagon-induced increases in cellular cAMP (b) or PKA activity (not shown). However, adrenalectomy did inhibit the stimulation by glucagon of GPase activity (c) and glucose output (not shown). Note that, in the sham-operated or untreated group, a concentration of 60 (10^2.79) pM near-maximally activated GPase (c), but had minimal effects on tissue cAMP levels (a and b). These results suggest that the “sensitizing” effects of exogenous corticosteroids are apparent ex vivo, but may not be consistently induced by endogenous corticosteroids in vivo (see Figures 7 and 8). As a control, endogenous corticosteroids do seem to contribute to the stimulation of phosphorylase activity by zone 1 and zone 2 concentrations of glucagon in vivo (c). Adapted from references Christoffersen et al. (1984) (a) and Chan et al. (1979) (b and c)
The current, AC/cAMP-based model of glucagon’s hepatic actions should be revised, and the focus of future research should be redirected accordingly, placing much greater emphasis on the role of the PLC/IP3 pathway.

10 CONCLUSIONS AND FUTURE INVESTIGATIONS

If there is one take-home message of this review, it is this: When it comes to investigating the endocrinology of glucagon, concentration matters. The weight of the evidence indicates that plasma glucagon concentrations are below 60 pM in the absence of metabolic stress or in T2D, and within that range do not activate hepatic AC above basal levels. When they rise above 100 pM in the early neonate or exercising adult, they predictably activate AC to adaptively boost glucose output to meet the elevated systemic glucose demand. In that regard, the adaptive response in exercising adults recapitulates that of the early neonate. Inconsistencies become apparent when glucagon concentrations are between 60 and 100 pM, characteristic of T1D and starvation. Activating AC by high, pharmacological concentrations—above 800 pM—produce responses that are not physiologically relevant and in any case are difficult to interpret because of extensive cross-talk between the two signal pathways and because the intracellular targets of the two pathways overlap considerably (Figure 11).

“Uncertainty” in the title of this review refers to a number of important unanswered questions, including: (1) What are the respective roles of the PLC/IP3 and AC/cAMP pathways in the regulation of glucose output by glucagon in the absence and presence of metabolic stress? (2) Exactly how low is the glucagon concentration in hepatic portal plasma, and how, precisely, does its concentration vary in response to various metabolically stressful conditions? (3) At what concentrations and to what extent does glucagon activate the hepatic PLC/IP3 pathway in the early neonate? (4) Which intracellular downstream targets are common, and which are unique, to the AC/cAMP and PLC/IP3 pathways? (5) As a target of the PLC/IP3 pathway, what is the role of AMPK in glucagon’s regulation of hepatic glucose mobilization in vivo? and (6) How do elevations in circulating glucocorticoids influence the regulation of hepatic glucose mobilization by glucagon under normal, metabolically-unstressed conditions, as well as in T1D or starvation?

Uncertainties remain, largely because focused experiments specifically designed to directly address the central questions posed in this review are rare. New studies blending classical pharmacological and modern molecular or gene manipulation approaches stand a good chance of filling at least some of the information gaps. For example, dose-response curves depicted in Figure 4 could be repeated, but employing techniques and experimental approaches that are extensions and refinements of those used by Sutherland and coworkers a half-century ago. The isolated perfused rat liver preparation that they and others have utilized can be replaced with an isolated mouse liver preparation, adapted for perfusion with glucose and fatty acids (Ferrigno et al., 2013; Harney & Rodgers, 2008), to allow the application of both pharmacological and gene manipulation techniques in the same study. If the portion of the glucagon-glucose mobilization curve that is below 60 pM shown in Figure 4 is displaced rightward by pretreatment with, for example, the PKA blocker H89 (Lochner & Moolman, 2006), by liver-specific PKA inhibition as discussed above (Willis et al., 2011), or by knocking out appropriate Gαs subtypes, then the results would support the alternative hypothesis that activation of the AC/cAMP pathway does contribute to the mediation of the glucose-mobilizing effects of glucagon at physiological concentrations in vivo. If, however, that portion of the curve is only affected by interventions such as pretreatment with the PLC antagonist U73122 or a selective Gq inhibitor such as GP2A or YM-19 (Zhang et al., 2020), or by knocking out the appropriate Gαq protein, then the results would support the central hypothesis proposed here, that glucagon regulates hepatic glucose mobilization exclusively via the PLC/IP3/CaM pathway at physiological concentrations. A glucagon-AC/cAMP dose-response curve like the one depicted in Figure 4 would serve as a control for both the effectiveness of anti-AC/cAMP interventions and the specificity of PLC or Gq inhibitors. Experiments of this kind would help to satisfy an unmet need in the characterization of glucagon’s true mechanism of action.

The longstanding cAMP bias should be seriously reexamined. It may have persisted this long because of the widespread reluctance to acknowledge the substantial gap between hepatic portal glucagon concentrations and the minimal concentration required to activate AC, along with an underappreciation of the efficacy and complexity
of the GR1/IP3/Ca\textsuperscript{2+}/CaM pathway (Figure 11). Too often in experimental reports or review articles, the PLC/IP3 pathway has been acknowledged only in passing or even ignored altogether (e.g., Christophe, 1995; Goldstein & Hager, 2018; Jiang & Zhang, 2003; Mauger et al., 1989; Rix et al., 2019; Schwartz et al., 1997; Wewer Albrechtsen et al., 2016), although there are welcome exceptions (e.g., Müller et al., 2017; Perry et al., 2020). In the future, more attention should be paid to the detailed characterization of the hepatic GR1 receptor complex and downstream
events, as well as to the interactions between the PLC/IP3 and AC/cAMP pathways, particularly at physiological and pathophysiologically relevant hormone concentrations. The administration of very high, pharmacological concentrations ex vivo, often at the extreme concentration of 100,000 pM, yields information that is applicable to the cellular consequences of a maximally activated AC/cAMP pathway, but generates intracellular responses of questionable relevance to the physiological or pathophysiological effects of the hormone on the liver in vivo. Given the increasing appreciation of glucagon’s central role in the etiology of diabetes (Burcelin et al., 1996; Johnson et al., 1982; Lee et al., 2012; Patil et al., 2020; Unger & Cherrington, 2012; Wewer Albrechtsen et al., 2019), resolving persistent uncertainties and establishing its true mechanism of action in health and disease are now more urgent than ever.1

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**ETHICS STATEMENT**

No studies on experimental animals or humans were carried out for this manuscript.

1For reviews on this and related topics, see Adeva-Andani et al. (2019), Agius (2007), Almahairi et al. (2014), Altarejos and Montminy (2011), Barthel and Schmoll (2003), Bill and Vines (2020), Blunosom and Cockroft (1865), Brushia and Walsh (1999), Burcelin et al. (1996), Campbell and Newgard (2021), Carling et al. (2011), Chin andMeans (2000), Christophe (1995, 1996), Cohen (1989), Cohen et al. (2015), D’Alesio (2011), Dunning and Gerich (2007), Engin et al. (2017), Exton (1979, 1986, 1987, 1993), Exton et al. (1981), Foretz et al. (2005), Fullerton (2016), Girard (1986, 1990), Güemes et al. (2016), Han et al. (2016), Hasenour et al. (2013), Hook and Means (2001), Jacques et al. (2018), Janah et al. (2020), Jiang and Zhang (2003), Jitrapakdee (2012), Kaira et al. (2021), Klein and Malviya (2008), Kraus-Friedmann (1984), Ktorza et al. (1985), Lin and Accili (2011), Mayo et al. (2003), Mayor and Cuezva (1985), Miller and Birnbaum (2016), Moore et al. (1998), Mortimore and Pissö (1987), Müller et al. (2017), Newton (2018), Nimmo and Cohen (1977), Nishizuka (1989), Okar et al. (2004), Ong and Ambudkar (2020), Patil et al. (2020), Pilkis and Ghranner (1992), Racioppo and Means (2012), Raju and Cryer (2005), Ravnkjær et al. (2015), Rider et al. (2004), Rix et al. (2019), Rodgers (2012, 2021), Rui (2014), Servillo et al. (2002), Sharabi et al. (2019), Soderling (1999), Sugden et al. (1993), Taborsky (2010), Unger (1985), Unger and Cherrington (2012), Unterman (2018), Viollet et al. (2009), Wendt and Ellassos (2020), Wewer Albrechtsen et al. (2016), Wicks (1971), Williamson et al. (1987), Winder (1985), Wu-Zhang and Newton (2013), Xing et al. (2018), Yabuhari and Bashyam (2010), Yang and Yang (2016) and Zhang, et al. (2019).

**CONFLICT OF INTEREST**

The author declares no conflict of interest.

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**REFERENCES**

Aalinkeel, R., Srinivasan, M., Kalhan, S. C., Laychock, S. G., & Patel, M. S. (1999). A dietary intervention (high carbohydrate) during the neonatal period causes islet dysfunction in rats. *American Journal of Physiology-Endocrinology and Metabolism*, 277(6), E1061–E1069. https://doi.org/10.1152/ajpendo.1999.277.6.E1061

Adeva-Andani, M. M., Funcasta-Calderón, R., Fernández-Fernández, C., Castro-Quintela, E., & Carnero-Freira, N. (2019). Metabolic effects of glucagon in humans. *Journal of Clinical & Translational Endocrinology* 15, 45–53. https://doi.org/10.1016/j.jcte.2018.12.005

Adigun, A. A., Wrench, N., Seidler, F. J., & SLOTKIN, T. A. (2010). Neonatal dexamethasone treatment leads to alterations in cell signaling cascades controlling hepatic and cardiac function in adulthood. *Neurotoxicology and Teratology*, 32, 193–199. https://doi.org/10.1016/j.ntt.2009.10.002

Aggarwal, S. R., Lindros, K. O., & Palmer, T. N. (1995). Glucagon stimulates phosphorylation of different peptides in isolated perportal and perivenous hepatocytes. *FEBS Letters*, 377, 439–443.

Agius, L. (2007). New hepatic targets for glycaemic control in diabetes. *Best Practice & Research Clinical Endocrinology & Metabolism*, 21, 587–605. https://doi.org/10.1016/j.beem.2007.09.001

Aguilar-Parada, E., Eisentraut, A. M., & Unger, R. H. (1969). Effects of starvation on plasma pancreatic glucagon in normal man. *Diabetes*, 18, 717–723. https://doi.org/10.2337/diab.18.11.717

Allgayer, H., Bachmann, W., & Hepp, K. D. (1982). Increased dose-response relationship of liver plasma membrane adenylate cyclase to glucagon stimulation in diabetic rats. A possible role of the guanyl nucleotide-binding regulatory protein. *Diabetologia*, 22, 464–467. https://doi.org/10.1007/BF00282591

Almahairi, M., Mei, F. C., & Cheng, X. (2014). cAMP sensor EPAC proteins and energy homeostasis. *Trends in Endocrinology and Metabolism*, 25, 60–71.

Altarejos, J., & Montminy, M. (2011). CREB and the CRTC coactivators: Sensors for hormonal and metabolic signals. *Nature Reviews Molecular Cell Biology*, 12, 141–151.

Anderson, K. A., Lin, F., Ribar, T. L., Stevens, R. D., Muehlbauer, M. J., Newgard, C. B., & Means, A. R. (2012). Deletion of CaMKK2 from the liver lowers blood glucose and improves whole-body glucose tolerance in the mouse. *Molecular Endocrinology*, 26, 281–291. https://doi.org/10.1210/me.2011-1299

Andersson, M., Carlquist, M., Maletti, M., & Marie, J.-C. (1993). Simultaneous solubilization of high-affinity receptors for VIP and glucagon and of a low-affinity binding protein for VIP, shown to be identical to calmodulin. *FEBS Letters*, 318, 35–40. https://doi.org/10.1016/0014-5793(93)81322-Q

Androgué, H. J., Chap, Z., Ishida, T., & Field, J. B. (1985). Role of the endocrine pancreas in the kalemic response to acute metabolic acidosis in conscious dogs. *Journal of Clinical Investigation*, 75, 798–808. https://doi.org/10.1172/JCI111775
Anyamaneeratch, K., Rojvirat, P., Sukjoi, W., & Jitrapakdee, S. (2015). Insights into the transcriptional regulation of hepatic glucose production. *International Review of Cell and Molecular Biology, 318*, 203–253.

Aromataris, E. C., Roberts, M. L., Barritt, G. J., & Rychkov, G. Y. (2006). Glucagon activates Ca2+ and Cl− channels in rat hepatocytes. *Journal of Physiology, 573*(3), 611–625.

Assimacopoulos-Jeannet, F. D., Blackmore, P. F., & Exton, J. H. (1977). Studies on α-activation of hepatic glucose output. *Journal of Biological Chemistry, 252*, 2662–2669.

Aw, D. K. L., Sinha, R. A., Xie, S. Y., & Yen, P. M. (2014). Differential AMPK phosphorylation by glucagon and metformin regulates insulin signaling in human hepatic cells. *Biochemical and Biophysical Research Communications, 447*, 569–573.

Back, D. W., Sohal, P. S., & Angel, J. F. (1985). Effects of diet and selected hormones on the activities of hepatic malic enzyme and glucose-6-phosphate dehydrogenase in infant, prematurely weaned rats. *Journal of Nutrition, 115*, 625–632.

Bak, M. J., Wewer Albrechtsen, N., Petersen, J., Hartmann, B., Christensen, M., Vilsboll, T., Knop, F. K., Deacon, C. F., Dragsted, L. O., & Holst, J. J. (2014). Specificity and sensitivity of commercially available assays for glucagon and oxyntomodulin measurement in humans. *European Journal of Endocrinology, 170*, 529–538. [https://doi.org/10.1530/EJE-13-0941](https://doi.org/10.1530/EJE-13-0941)

Balks, H.-J., & Jungermann, K. (1984). Regulation of peripheral insulin/glucagon levels by rat liver. *European Journal of Biochemistry, 141*, 645–650.

Baly, D. L., Keen, C. L., & Hurley, L. S. (1985). Pyruvate carboxylase and phosphoenolpyruvate carboxylase activity in developing rats: Effect of manganese deficiency. *Journal of Nutrition, 115*, 872–879.

Banzet, S., Koulmann, N., Simler, N., Sanchez, H., Chapot, R., Serrurier, B., Peinnequin, A., & Bigard, X. (2009). Control of gluconeogenic genes during intense/prolonged exercise: Hormone-independent effect of muscle-derived IL-6 on hepatic tissue and PEPCK mRNA. *Journal of Applied Physiology, 107*, 1830–1839.

Barthel, A., & Schmoll, D. (2003). Novel concepts in insulin regulation of hepatic gluconeogenesis. *American Journal of Physiology. Endocrinology and Metabolism, 285*, E685–E692. [https://doi.org/10.1152/ajpendo.00253.2003](https://doi.org/10.1152/ajpendo.00253.2003)

Bartlett, P. J., Gaspers, L. D., Pierobon, N., & Thomas, A. P. (2014). Calcium-dependent regulation of glucose homeostasis in the liver. *Cell Calcium, 55*, 306–316. [https://doi.org/10.1016/j.ceca.2014.02.007](https://doi.org/10.1016/j.ceca.2014.02.007)

Barucha, D. B., & Tager, H. S. (1990). Analysis of glucagon-receptor interactions on isolated canine hepatocytes. Formation of reversibly and irreversibly cell-associated hormone. *Journal of Biological Chemistry, 265*, 3070–3079. [https://doi.org/10.1016/S0021-9258(19)39735-2](https://doi.org/10.1016/S0021-9258(19)39735-2)

Batcher, E., Madaj, P., & Gianoukakis, A. G. (2011). Pancreatic neuroendocrine tumors. *Endocrine Research, 36*, 35–43. [https://doi.org/10.3109/07435800.2010.525085](https://doi.org/10.3109/07435800.2010.525085)

Baumann, G., Puuvilai, G., Freinkel, N., Domont, L. A., Metzger, B. E., & Leven, H. B. (1981). Hepatic insulin and glucagon receptors in pregnancy: their role in the enhanced catabolism during fasting. *Endocrinology, 108*, 1979–1986.

Beaudry, M. A., Chiasson, J. L., & Exton, J. H. (1977). Gluconeogenesis in the suckling rat. *American Journal of Physiology, 233*, E175–180.

Berger, C. M., Sharis, P. J., Bracy, D. P., Lacy, D. B., & Wasserman, D. H. (1994). Sensitivity of exercise-induced increase in hepatic glucose production to glucose supply and demand. *American Journal of Physiology (Endocrinology and Metabolism), 30*, 267, E411–E421.

Berglund, E. D., Lee-Young, R. S., Lustig, D. G., Lynes, S. E., Donahue, E. P., Camacho, R. C., Meredith, M. E., Magnuson, M. A., Charron, M. J., & Wasserman, D. H. (2009). Hepatic energy state is regulated by glucagon receptor signaling in mice. *Journal of Clinical Investigation, 119*, 2412–2422.

Bill, C. A., & Vines, C. M. (2020). Phospholipase C Advances in Experimental Medicine and Biology, 1131, 215–242.

Biondi, R., & Viola-Magni, M. P. (1977). Regulatory mechanisms of hepatic phosphorylase in fetal and neonatal livers of rats. *American Journal of Physiology-Endocrinology and Metabolism, 232*(4), E370–E374. [https://doi.org/10.1152/ajpendo.1977.232.4.E370](https://doi.org/10.1152/ajpendo.1977.232.4.E370)

Bizeau, M. E., & Hazel, J. R. (1999). Dietary fat type alters glucose metabolism in isolated rat hepatocytes. *Journal of Nutritional Biochemistry, 10*, 709–715. [https://doi.org/10.1016/S0955-2863(99)00060-1](https://doi.org/10.1016/S0955-2863(99)00060-1)

Blackmore, P. F., & Exton, J. H. (1986). Studies on the hepatic calcium-mobilizing activity of aluminum fluoride and glucagon. *Journal of Biological Chemistry, 261*, 11056–11063.

Blair, J. B., Cimbala, M. A., Foster, J. L., & Morgan, R. A. (1976). Hepatic pyruvate kinase. Regulation by glucagon, cyclic adenosine 3′-5′-monophosphate, and insulin in the perfused rat liver. *Journal of Biological Chemistry, 251*, 3756–3762. [https://doi.org/10.1016/S0021-9258(17)33408-7](https://doi.org/10.1016/S0021-9258(17)33408-7)

Blauw, H., Wendl, I., DeVries, J. H., Heise, T., & Jax, T. (2015). Pharmacokinetics and pharmacodynamics of various glucagon dosages at different blood glucose levels. *Diabetes, Obesity & Metabolism, 18*, 34–39. [https://doi.org/10.1111/dom.12571](https://doi.org/10.1111/dom.12571)

Blommaart, P. J. E., Charles, R., Meijer, A., & Lamers, W. H. (1995). Changes in hepatic nitrogen balance in plasma concentrations of amino acids and hormones in cell volume after overnight fasting in perinatal and adult rats. *Pediatric Research, 38*, 1018–1025.

Blunsom, N. J., & Cockcroft, S. (1865). Phosphatidylinositol synthesis at the endoplasmic reticulum. *Molecular and Cell Biology of Lipids, 1865*(1), 158471. [https://doi.org/10.1016/j.jbhipal.2019.05.015](https://doi.org/10.1016/j.jbhipal.2019.05.015)

Bois-Joyeux, B., Chanez, M., Azzout, B., & Peret, J. (1986). Comparison between starvation and consumption of a high protein diet: Plasma insulin and glucagon and hepatic activities of gluconeogenic enzymes during the first 24 hours. *Diabetes Metabolism (Paris), 12*, 22–37.

Bolli, G. B., Tsalkian, E., Haymond, M. W., Cryer, P. E., & Gerich, J. E. (1984). Defective glucose counterregulation after subcutaneous insulin in noninsulin-dependent diabetes mellitus. *Journal of Clinical Investigation, 73*, 1532–1541.

Bonnevie-Nielsen, V., & Tager, H. S. (1983). Glucagon receptors on isolated hepatocytes and hepatocyte membrane vesicles. *Journal of Biological Chemistry, 258*, 11313–11320.

Borghi, V. C., Wajchenberg, B. L., & Abuquerque, R. H. (1984). Evaluation of a sensitive and specific radioimmunoassay for pancreatic glucagon in human plasma and its clinical application. *Clinica Chimica Acta, 136*, 39–48. [https://doi.org/10.1016/0009-8981(84)90245-6](https://doi.org/10.1016/0009-8981(84)90245-6)

Brand, C. L., Jøgensen, P. N., Svendsen, I., & Holst, J. (1996). Evidence for a major role for glucagon in regulation of plasma...
glucose in conscious, nondiabetic, and alloxan-induced diabetic rabbits. Diabetes, 45, 076–1083. https://doi.org/10.2337/diabetes.45.8.1076

Brodows, R. G. (1985). Starvation enhances the ability of insulin to inhibit its own secretion. Metabolism, 24, 53–57. https://doi.org/10.1016/0026-0495(85)90060-5

Brushia, R. J., & Walsh, D. A. (1999). Phosphorylase kinase: The complexity of its regulation is reflected in the complexity of its structure. Frontiers in Bioscience, 4, 618–641.

Bruzowski, J. S., & Skelding, K. A. (2019). The multi-functional calcium/calmodulin stimulated protein kinase (CaMK) family: Emerging targets for anti-cancer therapeutic intervention. Pharmaceuticals (Basel), 12, 8. https://doi.org/10.3390/ph120810008

Buchanan, K. D., Vance, J. E., Dinstl, K., & Williams, R. H. (1969). Effect of glucose on glucagon secretion in anesthetized dogs. Diabetes, 18, 11–18.

Burcelin, R., Katz, E. B., & Charron, M. J. (1996). Molecular and cellular aspects of the glucagon receptor: Role in diabetes and metabolism. Diabetes & Metabolism (Paris), 22, 373–396.

Bygrave, F. L., & Benedetti, A. (1993). Calcium: Its modulation in liver by cross-talk between the actions of glucagon and calcium-mobilizing agonists. The Biochemical Journal, 296, 1–14.

Camici, M., Ahmad, Z., DePaoli-Roach, A. A., & Roach, P. J. (1984). Phosphorylation of rabbit liver glycogen synthase by multiple protein kinases. Journal of Biological Chemistry, 259, 2466–2473. https://doi.org/10.1016/S0021-9258(17)43376-X

Campbell, J. E., & Newgard, C. B. (201). Mechanisms controlling pancreatic islet cell function in insulin secretion. Nature Reviews Molecular Cell Biology, 22, 142–158.

Cánepe, E. T., Galvagno, M. A., Llambias, E. B. C., Passeron, S., & Grinstein, M. (1985). Studies on regulatory mechanisms in heme biosynthesis in hepatocytes from experimental diabetic rats. Biochimica Et Biophysica Acta, 847, 191–197.

Cánepe, E. T., Llambias, E. B. C., & Grinstein, M. (1990). Studies on induction of δ-aminolevulinic acid synthase, ferrochelatase, cytochrome P-450, and cyclic AMP by phenformin. Biochemical Pharmacology, 40, 365–372.

Cárdenas-Tanús, R. J., Huerta-Bahena, J., & García-Sáinz, A. (1982). Angiotensin II inhibits the accumulation of cyclic AMP produced by glucagon but not its metabolic effects. FEBS Letters, 143, 1–4.

Carling, D., Mayer, F. V., Sanders, M. J., & Gamblin, S. J. (2011). AMP-activated protein kinase: Nature’s energy sensor. Nature Chemical Biology, 7, 512–518.

Carlson, C. L., & Winder, W. W. (1999). Liver AMP-activated protein kinase and acetyl-CoA carboxylase activity during and after exercise. Journal of Applied Physiology, 86, 669–674.

Carlson, K. I., Marker, J. C., Aranall, D. A., Terry, M. L., Yang, H. T., Lindsay, L. G., Bracken, M. E., & Winder, W. W. (1985). Epinephrine is unessential for stimulation of liver glycogenolysis during exercise. Journal of Applied Physiology, 58, 544–548. https://doi.org/10.1152/jappl.1985.58.2.544

Caruana, J. A., Goldman, J. K., Camara, D. S., & Gage, A. A. (1981). Insulin, glucagon, and glucose in the regeneration response of the liver. Surgery, Gynecology and Obstetrics, 153, 726–730.

Casteleijn, E., van Rooij, H. C. J., van Berkel, T. J. C., & Koster, J. F. (1986). Mechanism of glucagon stimulation of fructose-1,6-bisphosphatase in rat hepatocytes. FEBS Letters, 201, 193–197. https://doi.org/10.1016/0014-5793(86)80607-X

Cersosimo, E., Williams, P., Geer, R., Lairmore, T., Ghishan, F., & Abumrad, N. J. (1989). Importance of ammonium ions in regulating hepatic glutamine synthesis during fasting. American Journal of Physiology-Endocrinology and Metabolism, 257(4), E514–519. https://doi.org/10.1152/ajpendo.1989.257.4.E514

Cersosimo, E., Zaitseva, I. N., & Ajmal, M. (1998). Effects of β-adrenergic blockade in hepatic and renal glucose production during hypoglycemia in conscious dogs. American Journal of Physiology, 275, E792–E797.

Chamras, H., Fouchereau-Peron, M., & Rosselin, G. (1980). The effect of streptozotocin-induced diabetes on the early steps of glucagon action in isolated rat liver cells. Diabetologia, 19, 74–80. https://doi.org/10.1007/BF00258315

Chan, T. M., Steiner, K. E., & Exton, J. H. (1979). Effects of adrenalectomy on hormone action on hepatic glucose metabolism. Journal of Biological Chemistry, 254, 11374–11378.

Charbonneau, A., Melancon, A., Lavole, C., & Lavole, J.-M. (2005). Alterations in hepatic glucagon receptor density and in Gsα and Gα2 protein content with diet-induced hepatic steatosis: effects of acute exercise. American Journal of Physiology, 289, E8–E14.

Charest, R., Prpić, V., Exton, J. H., & Blackmore, P. F. (1985). Stimulation of inositol triphosphate formation in hepatocytes by vasopressin, adrenaline, and angiotensin II and its relationship to changes in cytosolic free calcium. The Biochemical Journal, 227, 79–90.

Chen, M., Gavrilova, O., Zhao, W.-Q., Nguyen, A., Lorenzo, J., Shen, L., Nackers, L., Pack, S., Jou, W., & Weinstein, L. S. (2005). Increased glucose tolerance and reduced adiposity in the absence of fasting hypoglycemia in mice with liver-specific Gα deficiency. Journal of Clinical Investigation, 115, 3217–3227. https://doi.org/10.1172/JCI24196

Chin, D., & Means, A. R. (2000). Calmodulin: a prototypical calcium sensor. Trends in Cell Biology, 10, 322–328.

Christ, B., Nath, A., & Jungermann, K. (1990). Mechanism of the inhibition by insulin of the glucagon-dependent activation of the phosphoenolpyruvate carboxykinase gene in rat hepatocyte cultures. Action on gene transcription, mRNA level and -stability as well as hysteresis effect. Biological Chemistry Hoppe-Seyler, 371, 395–402.

Christoffersen, T., Refsnes, M., Bronstad, G. O., Østby, E., Huse, J., Haffner, F., Sand, T.-E., Hunt, N. H., & Sonne, O. (1984). Changes in hormone responsiveness and cyclic AMP metabolism in rat hepatocytes during primary cultures and effects of supplementing the medium with insulin and dexamethasone. European Journal of Biochemistry, 138, 217–226.

Christophe, J. (1995). Glucagon receptors: from genetic structure and expression to effector coupling and biological responses. Biochimica Et Biophysica Acta, 1241, 45–57.

Christophe, J. (1996). Glucagon and its receptor in various tissues. Annales of the New York Academy of Sciences, 805, 31–42.

Ciprés, G., Buttqa, N., Urbela, E., Parrilla, R., & Martin-Quegeo, A. (1995). Impaired protein kinase C activation is associated with decreased hepatic alpha-1 adrenocceptor responsiveness in adenoclanotomic rats. Endocrinol, 136, 468–475.

Ciudad, C., Camici, M., Ahmad, Z., Wang, Y., DePaoli-Roach, A. A., & Roach, P. J. (1984). Control of glycosen synthase phosphorolization in isolated rat hepatocytes by epinephrine, vasopressin and glucagon. European Journal of Biochemistry, 142, 511–520.

Claeyssens, M. W., Lavonne, A., Vaillant, C., Rakotomanga, J. A., Bois-Jooyeu, B., & Peret, J. (1992). Metabolic changes during
early starvation in rats fed a low-protein diet in the post-weaning growth period. *Metabolism, 41*, 722–727. https://doi.org/10.1016/0026-0495(92)90311-W

Clark, M. G., & Jarrett, I. G. (1978). Responsiveness to glucagon by isolated rat hepatocytes controlled by the redox state of the cytosolic nicotinamide-adenine dinucleotide couple acting on adenosine 3′:5′-cyclic monophosphate phosphodiesterase. *The Biochemical Journal, 176*, 805–816.

Claus, T. H., El-Maghrabi, M. R., & Pilkis, S. J. (1979). Modulation of the phosphorylation state of rat liver pyruvate kinase by allosteric effectors and insulin. *Journal of Biological Chemistry*, 254, 7855–7864. https://doi.org/10.1016/S0021-9258(18)36025-3

Cohen, P. (1989). The structure and regulation of protein phosphatases. *Annual Review of Biochemistry, 58*, 453–508. https://doi.org/10.1146/annurev.bi.58.070189.002321

Cohen, S., Li, B., Tsiens, R. W., & Ma, H. (2015). Evolutionary and functional perspectives on signaling from neuronal surface to nucleus. *Biochemical and Biophysical Research Communications, 460*, 88–99. https://doi.org/10.1016/j.bbrc.2015.02.146

Coker, R. H., Koyama, Y., Brooks, D., Williams, P. E., Rheaume, N., & Wasserman, D. H. (1999). Splanchnic glucagon kinetics in exercising alloxaan-diabetic dogs. *Journal of Applied Physiology, 86*, 1626–1631. https://doi.org/10.1152/jappl.1999.86.5.1626

Coker, R. H., Koyama, Y., Lacy, D. B., Williams, P. E., Rheaume, N., & Wasserman, D. H. (1999). Pancreatic innervation is not essential for exercise-induced changes in glucagon and insulin or glucose kinetics. *American Journal of Physiology, 277*, E1122–E1129. https://doi.org/10.1152/ajpendo.1999.277.6.E1122

Connelly, P. A., Sisk, R. B., Schulman, H., & Garrison, J. C. (1987). Evidence for the activation of the multifunctional Ca2+/calmodulin-dependent protein kinase in response to hormones that increase intracellular Ca2+. *Journal of Biological Chemistry*, 262, 10154–10163.

Corvera, S., & García-Sáinz, J. A. (1984). Phorbol esters inhibit alpha, adrenergic stimulation of glycogenolysis in isolated rat hepatocytes. *Biochemical and Biophysical Research Communications, 119*, 1128–1133.

Corvera, S., Huerta-Bahena, H., Pelton, J. T., Hruby, V. J., Trivedi, D., & García-Sáinz, J. A. (1984). Metabolic effects and cyclic AMP levels produced by glucagon, [1-Na-trinitrophenylhistidine, 12-homoarginine] glucagon and forskolin in isolated rat hepatocytes. *Biochimica Et Biophysica Acta, 804*, 434–441.

Cruces-Sande Arcones, A. C., Vila-Bedmar, R., Val-Blasco, A., Sharabi, K., Díaz-Rodriguez, D., Puigserver, P., Mayor, F. J., & Murga, C. (2020). Autophagy mediates hepatic GRK2 degradation to facilitate glucagon-induced metabolic adaptation to fasting. *The FASEB Journal, 34*, 399–409. https://doi.org/10.1096/fj.20190444R

Curnow, R. T., Rayfield, E. J., George, D. T., Zenser, T. V., & DeRubertis, F. R. (1976). Altered hepatic glycogen metabolism and glucoheparyulatory hormones during sepsis. *American Journal of Physiology*, 230, 1296–1301. https://doi.org/10.1152/ajplegacy.1976.230.5.1296

D’Alessio, D. (2011). The role of dysregulated glucagon secretion in Type 2 diabetes. *Diabetes, Obesity and Metabolism, 13*(Suppl 1), 126–132. https://doi.org/10.1111/j.1463-1326.2011.01449.x

Deacon, C. F., Kelstrup, M., Trebbien, R., Klarskov, L., Olesen, M., & Holst, J. J. (2003). Differential regional metabolism of glucagon in anesthetized pigs. *American Journal of Physiology-Endocrinology and Metabolism, 285*(3), E552–E560. https://doi.org/10.1152/ajpendo.00125.2003

Decaux, J.-F., Ferré, P., & Girard, J. (1986). Effect of weaning on different diets on hepatic gluconeogenesis in the rat. *Biology of the Neonate, 50*, 331–336. https://doi.org/10.1159/000242617

Demigné, C., Fafournoux, P., & Rémésy, C. (1985). Enhanced uptake of insulin and glucagon by liver in rats adapted to a high protein diet. *Journal of Nutrition, 115*, 1065–1072.

Dich, J., & Glud, C. N. (1976). Effect of glucagon on cyclic AMP, albumin metabolism and incorporation of 14C-leucine into proteins in isolated parenchymal rat liver cells. *Acta Physiologica Scandinavica, 97*, 457–469.

Dighe, R. R., Rojas, F. J., Birnbaumer, L., & Garber, A. J. (1984). Glucagon-stimulable adenylyl cyclase in rat liver. The impact of streptozotocin-induced diabetes mellitus. *Journal of Clinical Investigation, 73*, 1013–1023.

Dobbins, R. L., Davis, S. N., Neal, D. W., Cobelli, C., Jaspan, J., & Cherrington, A. D. (1995). Compartmental modeling of glucagon kinetics in the conscious dog. *Metabolism, 44*, 452–459.

Doi, Y., Iwai, M., Matsura, B., & Onji, M. (2001). Glucagon attenuates the action of insulin on glucose output in the liver of the Goto-Kakizaki rat perfused in situ. *Foliae Arboris - European Journal of Physiology, 442*, 537–541.

Dunning, B. E., & Gerich, J. E. (2007). The role of α-cell dysregulation on fasting and postprandial hyperglycemia in Type 2 diabetes and therapeutic implications. *Endocrine Reviews, 28*, 253–283.

Ekdahl, K. N., & Ekman, P. (1987). Effects of epinephrine, glucagon and insulin on the activity and degree of phosphorylation of fructose-1,6-bisphosphatase in cultured hepatocytes. *Biochimica Et Biophysica Acta, 929*, 318–326. https://doi.org/10.1016/0167-4889(87)90259-X

El-Maghrabi, M., Claus, T. H., Pilkis, J., Fox, E., & Pilkis, S. J. (1982). Regulation of rat liver fructose-2,6-bisphosphatase. *Journal of Biological Chemistry, 257*, 7603–7607. https://doi.org/10.1016/S0021-9258(18)34422-3

Engin, A. (2017). Human protein kinases and obesity. In A. Engin, & A. Engin, (eds). *Obesity and lipotoxicity. Advances in experimental medicine and biology*. Springer International.

England, R. D., Jenkins, W. T., Flanders, K. C., & Gurd, R. S. (1983). Noncooperative receptor interactions of glucagon and eleven analogues: Inhibition of adenylate cyclase. *Biochemistry, 22*, 1722–1728.

Erion, D. M., Kotas, M. A., McGlashon, J., Yonemitsu, S., Hsiao, J. J., Nagai, Y., Iwaski, T., Murray, S. F., Bhanot, S., Cline, G. W., Samuel, V. T., Shulman, G. I., & Gillum, M. P. (2013). cAMP-responsive element-binding protein (CREB)-regulated transcription coactivator 2 (CRTC2) promotes glucagon clearance and hepatic amino acid catabolism to regulate glucose homeostasis. *Journal of Biological Chemistry, 288*, 16167–16176. https://doi.org/10.1074/jbc.M113.460246

Evans, M. L., Hopkins, D., Macdonald, I. A., & Amiel, S. A. (2004). Alanine infusion during hypoglycaemia partly supports cognitive performance in healthy human subjects. *Diabetic Medicine, 21*, 440–446. https://doi.org/10.1111/j.1464-5491.2004.01174.x

Exton, J. H. (1979). Regulation of gluconeogenesis by glucocorticoids. *Monographs on Endocrinology, 12*, 535–546.

Exton, J. H. (1986). Mechanisms involved in calcium-mobilizing agonist responses. *Advanced Cyclic Nucleotides, Protein Phosphorylation Research, 20*, 211–262.
Exton, J. H. (1987). Mechanisms of hormonal regulation of hepatic glucose metabolism. *Diabetes/Metabolism Reviews*, 3, 163–183. https://doi.org/10.1002/dmr.5610030108

Exton, J. H. (1993). Role of G proteins in activation of phosphoinositide phospholipase C. *Advanced Section Mess Phosphoprotein Research*, 28, 65–72.

Exton, J. H., Blackmore, P. F., El-Refaei, M. F., Dehaye, J.-P., Strickland, W. G., Cherrington, A. D., Chan, T. M., Assimacopoulos-Jeannet, F. D., & Chrisman, T. D. (1981). Mechanism of hormonal regulation of liver metabolism. *Advances in Cyclic Nucleotide Research*, 14, 491–505.

Exton, J. H., Friedmann, N., Wong, E.-H.-A., Brineaux, J. P., Corbin, J. D., & Park, C. R. (1972). Interaction of glucocorticoids with glucagon and epinephrine in the control of gluconeogenesis and glycogenolysis in liver and of lipolysis in adipose tissue. *Journal of Biological Chemistry*, 247, 3579–3588. https://doi.org/10.1016/S0021-9258(19)45180-6

Exton, J. H., Lewis, S. B., Ho, R. J., Robison, G. A., & Park, C. R. (1971). The role of cyclic AMP in the interaction of glucagon and insulin in the control of liver metabolism. *Annals of the New York Academy of Sciences*, 185, 85–100.

Exton, J. H., Robison, G. A., Sutherland, E. W., & Park, C. R. (1971). Studies on the role of adenosine 3’-5’-monophosphate in the hepatic actions of glucagon and catecholamines. *Journal of Biological Chemistry*, 246, 6166–6177. https://doi.org/10.1016/S0021-9258(18)61771-5

Feliciu, J. E., Hue, L., & Hers, H.-G. (1976). Hormonal control of pyruvate kinase activity and of gluconeogenesis in isolated hepatocytes. *Proceedings of the National Academy of Sciences of the United States of America*, 73, 2762–2766. https://doi.org/10.1073/pnas.73.8.2762

Feriód, C. N., Nguyen, L., Jurczak, M. J., Kruglov, E. A., Nathanson, M. H., Shulman, G. I., Bennett, A. M., & Ehrlich, B. E. (2014). Inositol-1,4,5-triphosphate receptor type II (InsP3RII) is reduced in obese mice, but metabolic homeostasis is preserved in mice lacking InsP3R1L. *American Journal of Physiology: Endocrinology and Metabolism*, 307, E1057–E1064.

Fernández-Milán, E., de Toro-Martín, J., Lizárraga-Molinedo, E., Escriví, F., & Álvarez, C. (2013). Role of endogenous IL-6 in the neonatal expansion and functionality of Wistar rat pancreatic alpha cells. *Diabetologia*, 56, 1098–1107. https://doi.org/10.1007/s00125-013-2862-8

Ferrigno, A., Richelmi, P., & Vairetti, M. (2013). Troubleshooting and improving the mouse and rat isolated perfused liver preparation. *Journal of Pharmacological and Toxicological Methods*, 67, 107–114. https://doi.org/10.1016/j.vascn.2012.10.001

Fischer, L., Haag-Diergarten, S., Scharrer, E., & Lutz, T. A. (2005). Leukotriene and purinergic receptors are involved in the hyperpolarizing effect of glucagon in liver cells. *Biochimica Et Biophysica Acta*, 1669, 26–33. https://doi.org/10.1016/j.bbamem.2005.01.010

Fleig, W. E., Noether-Fleig, G., Roeben, H., & Ditschuneit, H. (1984). Hormonal regulation of key gluconeogenic enzymes and glucose release in cultured hepatocytes: effects of dexamethasone and gastrointestinal hormones on glucagon action. *Archives of Biochemistry and Biophysics*, 229, 368–378.

Foretz, M., Ancellin, N., Andreelli, F., Saintillan, Y., Grondin, P., Kahn, A., Thorens, B., Vaulont, S., & Viollet, B. (2005). Short-term overexpression of a constitutively active form of AMP-activated protein kinase in the liver leads to mild hypoglycemia and fatty liver. *Diabetes*, 54, 1331–1339. https://doi.org/10.2337/diabetes.54.5.1331

Foretz, M., Guigas, B., & Viollet, B. (2019). Understanding the gluco-regulatory mechanisms of metformin in type 2 diabetes. *Nature Reviews Endocrinology*, 10, 569–589.

Francavilla, A., Jones, A. F., Benichou, J., & Starzl, T. E. (1980). The effect of portacaval shunt upon hepatic cholesterol synthesis and cyclic AMP in dogs and baboons. *Journal of Surgical Research*, 28, 1–7. https://doi.org/10.1016/0022-4804(80)90074-8

Fries, J. L., Murphy, W. A., Sueiras-Diaz, J., & Coy, D. H. (1982). Somatostatin antagonist analog increases GH, insulin, and glucagon release in the rat. *Peptides*, 3, 811–814. https://doi.org/10.1016/0196-9781(82)90020-1

Fujita, Y., Gotto, A. M., & Unger, R. H. (1975). Basal and postprandial insulin and glucagon levels during a high and low carbohydrate intake and their relationships to plasma triglycerides. *Diabetes*, 24, 552–558. https://doi.org/10.2337/diab.diab.24.6.552

Fujisawa, Y., Kawaguchi, Y., Fujimoto, T., Kanayama, N., Magari, M., & Tokumitsu, H. (2016). Differential AMP-activated protein kinase (AMPK) recognition mechanism of Ca2+/calmodulin-dependent protein kinase isozymes. *Journal of Biological Chemistry*, 291, 13802–13808. https://doi.org/10.1074/jbc.M116.727867

Fullerton, M. D. (2016). AMP-activated protein kinase and its multifaceted regulation of hepatic metabolism. *Current Opinion in Lipidology*, 27, 172–180. https://doi.org/10.1097/MOL.0000000000000273

Furuya, E., Yokoyama, M., & Uyeda, K. (1982). Regulation of fructose-6-phosphate 2-kinase by phosphorylation and dephosphorylation: Possible mechanism of coordinated control by glycolysis and glycogenolysis. *Proceedings of the National Academy of Sciences*, 79, 325–329.

Gabby, R. A., & Lardy, H. A. (1984). Site of insulin inhibition of cAMP-stimulated glycogenolysis. *Journal of Biological Chemistry*, 259, 6052–6056. https://doi.org/10.1016/S0021-9258(20)82102-4

Galsgaard, K. D., Pedersen, J., Knop, F. K., Holst, J. J., & Weyer Albrechtsen, N. J. (2019). Glucagon and receptor signaling in lipid metabolism. *Frontiers in Physiology*, 10, 1–11.

Gannon, M. C., & Nuttall, F. Q. (1993). Physiological doses of oral casein affect hepatic glycogen metabolism in normal food-deprived rats. *Journal of Nutrition*, 125, 1159–1166.

Gawler, D. J., Milligan, G., Speigel, A. C., Unson, C. G., & Houslay, M. D. (1987). Abolition of the expression of inhibitory guanine nucleotide regulatory protein G i activity in diabetes. *Nature*, 327, 229–232. https://doi.org/10.1038/327229a0

Gehrand, A. L., Hoeynck, B., Jablonski, M., Leonovicz, C., Ye, R., Scherer, P. E., & Raff, H. (2016). Sex differences in adult rat insulin and glucose responses to arginine: programming effects of neonatal separation, hypoxia, and hypothermia. *Physiological Reports*, 4, e12972. https://doi.org/10.14814/phy2.12972

Gelling, R. W., Du, X. Q., Dickmann, D. S., Romer, J., Huang, H., Cui, L., Obici, S., Tang, B., Holst, J. J., Fledelius, C., Johansen, P. B., Rossetti, L., Jelicks, L. A., Serum, P., Nishimura, E., & Charron, M. J. (2003). Lower blood glucose, hyperglucagonemia, and pancreatic alpha cell hyperplasia in glucagon receptor knock-out mice. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 1438–1443.

Girard, J. (1986). Gluconeogenesis in late fetal and early neonatal life. *Biology of the Neonate*, 50, 237–258. https://doi.org/10.1159/000242605
Goldstein, D. E., & Curnow, R. T. (1978). Effect of starvation on hepatic glycogen metabolism and glucose homeostasis. *Metabolism*, 27, 315–323.

Goldstein, D. E., Sutherland, C. A., & Curnow, R. T. (1978). Altered mechanism of glucagon-mediated hepatic glycogenolysis during long-term starvation in the rat. *Metabolism*, 27, 1491–1497. https://doi.org/10.1016/S0026-0495(78)80021-3

Goldstein, I., & Hager, G. L. (2018). The three Ds of transcription activation by glucagon: Direct, delayed, and dynamic. *Endocrinology*, 159, 206–216.

Gosmanov, N. R., Szoke, E., Israelian, Z., Smith, T., Cryher, P. E., Gerich, J. E., & Meyer, C. (2005). Role of the decrement in intraslit insulin for the glucagon response to hypoglycemia in humans. *Diabetes Care*, 28, 1124–1131. https://doi.org/10.2337/diabetes.28.5.1124

Green, A. D., Vasu, S., & Flatt, P. R. (2016). Functionality and antidiabetic utility of β- and L-cell containing pseudosilts. *Experimental Cell Research*, 344, 201–209. https://doi.org/10.1016/j.yexcr.2016.04.007

Güemes, M., Rahman, S. A., & Hussain, K. (2016). What is normal blood glucose? *Archives of Disease in Childhood*, 101, 569–574.

Hahn, P., Girard, J., Assan, R., Frohlich, J., & Kervran, A. (1977). Control of blood cholesterol levels in suckling and weaning rats. *Journal of Nutrition*, 107, 2062–2066.

Hahn, P., & Hassanali, S. (1982). The effect of 3,5,3′-triiodothyronine on phosphoenolpyruvate carboxykinase, fatty acid synthase, and malic enzyme activity of liver and brown fat of fetal and neonatal rats. *Biology of the Neonate*, 41, 1–7.

Hamaguchi, T., Fukushima, H., Uehara, M., Wada, S., Shirotani, T., Kishikawa, H., Ichinose, K., Yamaguchi, K., & Shichiri, M. (1991). Abnormal glucagon response to arginine and its normalization in obese hyperinsulinemic patients with glucose intolerance: Importance of insulin action on pancreatic alpha cells. *Diabetologia*, 34, 801–806.

Han, H. S., Kang, G., Kim, J. S., Choi, B. H., & Koo, S.-H. (2016). Regulation of glycose metabolism from a liver-centric perspective. *Experimental & Molecular Medicine*, 48, e218. https://doi.org/10.1038/emm.2015.122

Hansen, B. C., Jen, K. C., Pek, S. B., & Wolfe, R. A. (1982). Rapid oscillations in plasma insulin, glucagon, and glucose in obese and normal weight humans. *Journal of Clinical Endocrinology and Metabolism*, 54, 785–792. https://doi.org/10.1210/jcem-54-4-785

Hansen, L. H., Gritomada, J., Bouchelouche, P., Whitmore, T., Jelinek, L., Kindsvogel, W., & Nishimura, E. (1998). Glucagon-mediated Ca2+ signaling in BHK cells expressing cloned human glucagon receptors. *American Journal of Physiology*, 274, C1552–C1562.

Hansen, R. W., & Reshef, L. (1997). Regulation of phosphoenolpyruvate carboxykinase (GTP) gene expression. *Annual Review of Biochemistry*, 66, 581–611.

Harney, J. A., & Rodgers, R. L. (2008). Insulin-like stimulation of cardiac fuel metabolism by physiological levels of glucagon: Involvement of PI3K but not cAMP. *American Journal of Physiology*, 295, E155–E161.

Hasenour, C. M., Berglund, E. D., & Wasserman, D. H. (2013). Emerging role of AMP activated protein kinase in endothrine control of metabolism in the liver. *Molecular and Cellular Endocrinology*, 366, 152–162. https://doi.org/10.1016/j.mce.2012.06.018

Hatting, M., Tavares, C. D. J., Shariabi, K., Rines, A. K., & Puigserver, P. (2018). Insulin regulation of gluconeogenesis. *Annals of the New York Academy of Sciences*, 1411, 21–35.

Hawdon, J. M., Aynsley-Green, A., Bartlett, K., & Ward Platt, M. P. (1993). The role of pancreatic insulin secretion in neonatal glucoregulation. II. Infants with disordered blood glucose homeostasis. *Archives of Disease in Childhood*, 68, 280–285.

Hawdon, J. M., Weddell, A., Aynsley-Green, A., & Ward Platt, M. P. (1993). Hormonal and metabolic response to hypoglycemia in small for gestational age infants. *Archives of Disease in Childhood*, 68, 269–273.

Heise, T., Heinemann, L., Heller, S., Weyer, Y. W., Strobel, S., Koltermann, O., & Maggs, D. (2004). Effect of pramlintide on symptom, catecholamine, and glucagon responses to hypoglycemia in healthy subjects. *Metabolism*, 53, 1227–1232. https://doi.org/10.1016/j.metabol.2004.04.010

Henkel, E., Menschikowski, M., Koehler, C., Leonhardt, W., & Hanefeld, M. (2005). Impact of glucagon response on postprandial hyperglycaemia in men with impaired glucose tolerance and type 2 diabetes mellitus. *Metabolism*, 54, 1168–1173. https://doi.org/10.1016/j.metabol.2005.03.024

Hermida, O. G., Fontela, T., Ghiglione, M., & Utenthal, L. O. (1994). Effect of lithium on plasma glucose, insulin and glucagon in normal and streptozotocin-diabetic rats: Role of glucagon in the hyperglycaemic response. *British Journal of Pharmacology*, 111, 861–865.

Hermensdorf, T., Dettmer, D., & Hofmann, E. (1989). Age-dependent effects of phorbol ester on adenylyl cyclase stimulation by glucagon in liver of female rats. *Biomedica Biochimica Acta*, 48, 255–260.

Hernandez, E., Leite, M. F., Guerra, M. T., Kruglov, E. A., Bruna-Romero, O., Rodrigues, M. A., Gomes, D. A., Giordano, F. J., Dranoff, J. A., & Nathanson, M. H. (2007). The special distribution of inositol 1,4,5-trisphosphate receptor isoforms shapes calcium waves. *Journal of Biological Chemistry*, 282, 10057–10067.

Herzig, S., Long, F., Jhala, U. S., Hedrick, S., Quinn, R., Bauer, A., Rudolph, D., Schutz, G., Yoon, C., Puigserver, P., Spiegelman, B., & Montminy, M. (2001). CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature*, 413, 179–183. https://doi.org/10.1038/35093131

Hespel, U., Jungermann, K., & Pöschel, G. P. (1995). Feedback-inhibition of glucagon-stimulated glycogenolysis in hepato-cyte/Kupffer cell cocultures by glucagon-elicited prostaglandin production in Kupffer cells. *Hepatology*, 22, 1577–1583. https://doi.org/10.1002/hep.1840220534

Heyworth, C. M., & Houslay, M. D. (1983). Insulin exerts actions through a distinct species of guanine nucleotide regulatory protein: Inhibition of adenylyl cyclase. *The Biochemical Journal*, 214, 547–552.

Heyworth, C. M., Wallace, A. V., & Houslay, M. D. (1983). Insulin and glucagon regulate the activation of two distinct membrane membrane-bound cyclicAMP phosphodiesterases in hepatocytes. *The Biochemical Journal*, 214, 99–110.
Hickman, R., Tyler, M., Innes, C. R., Lotz, Z., & Fourie, J. (1992). How rapidly do hyperinsulinemia and hyperglucagonemia develop after portocaval shunting? *Journal of Surgical Research*, 53, 20–23.

Higashi, K., & Hoek, J. B. (1991). Ethanol causes desensitization of receptor-mediated phospholipase C activation in isolated hepatocytes. *Journal of Biological Chemistry*, 266, 2178–2190.

Hirata, K., Pusl, T., O’Neill, A. F., Dranoff, J. A., & Nathanson, M. H. (2002). The type 2 inositol 1,4,5-triphosphate receptor can trigger Ca2+ waves in rat hepatocytes. *Gastroenterology*, 122, 1088–1100.

Holst, J. J. (1983). Gut glucagon, enteroglucagon, gut glucagon-like immunoreactivity, clefticin-current status. *Gastroenterology*, 84, 1602–1613.

Holst, J. J. (2014). Specificity and sensitivity of commercially available assays for glucagon and oxyntomodulin measurements in humans. *European Journal of Endocrinology*, 170, 529–538.

Holst, J. J., Burcharth, F., & Kühl, C. (1980). Pancreatic glucoregulatory hormones in cirrhosis of the liver: Portal vein concentrations during intravenous glucose tolerance test and in response to a meal. *Diabetes & Metabolism (Paris)*, 6, 117–127.

Hook, S. S., & Means, A. R. (2001). Ca2+-CaM-dependent protein kinases: From activation to function. *Annual Review of Pharmacology and Toxicology*, 41, 471–505.

Horikawa, S., Ishida, T., Iwaga, K., Kawanishi, K., Hartley, C. J., & Takahara, J. (1998). Both positive and negative portal venous and hepatic arterial glucose gradients stimulate hepatic glucose uptake after the same amount of glucose is infused into the splanchnic bed in conscious dogs. *Metabolism*, 47, 1295–1302.

House, P. D. R., Poulios, P., & Weidemann, M. J. (1972). Isolation of a plasma-membrane subtraction from rat liver containing an insulin-sensitive cyclic-AMP phosphodiesterase. *European Journal of Biochemistry*, 24, 429–437. https://doi.org/10.1111/j.1432-1033.1972.tb19703.x

Houslay, M. D. (1990). The use of selective inhibitors and computer modelling to evaluate the role of specific high affinity cyclic AMP phosphodiesterases in the hormonal regulation of hepatocyte intracellular cyclic AMP concentrations. *Cellular Signaling*, 2, 85–98. https://doi.org/10.1016/0898-6568(90)90036-A

Huang, O., Timofeeva, E., & Richard, D. (2006). Corticotropin-releasing factor and its receptors in the brain of rats with insulin and corticosterone deficits. *Journal of Molecular Endocrinology*, 37, 213–226. https://doi.org/10.1677/jme.1.02103

Hughes, B. P., Rye, K. A., Pickford, L. B., Barritt, G. J., & Chalmers, A. H. (1984). A transient increase in diacylglycerols is associated with the action of vasopressin on hepatocytes. *The Biochemical Journal*, 222, 535–540. https://doi.org/10.1042/bj2220535

Hume, R., McGeechan, A., & Burchel, A. (2002). Developmental disorders of glucose metabolism in infants. *Child: Care, Health and Development*, 28(Suppl 1), 45–47. https://doi.org/10.1046/j.1365-2214.2002.00013.x

Hussein, M. N., Kikuchi, K., Cukerman, E., Sirek, A., & Sirek, O. V. (1986). The effect of β-endorphin on biogenic amines, insulin, and glucagon levels in the hepatic portal circulation of normal and pancreatectomized dogs. *Endocrinol*, 119, 685–690.

Ichikawa, R., Takano, K., Fujimoto, K., Motomiya, T., Kobayashi, M., Kitamura, T., & Shichiri, M. (2019). Basal glucagon hypersecretion and response to oral glucose load in prediabetes and mild type 2 diabetes. *Endocrine J*, 66, 663–675. https://doi.org/10.1507/endocrj.EJ18-0372

Igumenova, T. I. (2015). Dynamics and membrane interactions of protein kinase C. *Biochemistry*, 54, 4953–4968. https://doi.org/10.1021/acs.biochem.5b00565

Ikezawa, Y., Yamatani, K., Ogawa, A., Ohnuma, H., Igarashi, M., Daimon, M., Manaka, H., & Sasaki, H. (1998). Effects of glucagon on glycogenolysis and gluconeogenesis are region-specific in periportal and perivenous hepatocytes. *Journal of Laboratory and Clinical Medicine*, 132, 547–555.

Illiano, G., & Cuatrececasas, P. (1972). Modulation of adenylate cyclase activity in liver and fat cell membranes by insulin. *Science*, 175, 906–908. https://doi.org/10.1126/science.175.4024.906

Imai, S., Fujita, K., Miura, M., Saeki, T., Kotaru, M., & Iwami, K. (2003). Postprandial changes in portal venous free amino acids and insulin/glucagon ratios as the result of protein over-intake are not directly linked to serine dehydratase induction in rat liver irrespective of age. *Journal of Nutritional Science and Vitaminology*, 247–255. https://doi.org/10.1777/jsmv.49.247

Imazu, M., Strickland, W. G., Chrisman, T. D., & Exton, J. H. (1984). Phosphorylation and inactivation of liver glycogen synthase by liver protein kinases. *Journal of Biological Chemistry*, 259, 113–1821. https://doi.org/10.1016/S0021-9258(17)43481-8

Ishida, T., Chou, M. C. Y., Lewis, R. M., Hartley, C. J., Entman, M., & Field, J. B. (1981). Effect of tolbutamide on hepatic extraction of insulin and glucagon and hepatic glucose output in anesthetized dogs. *Endocrinol*, 109, 443–450.

Ishida, T., Chap, Z., Chou, J., Lewis, R., Hartley, C., Entman, M., & Field, J. B. (1983). Differential effects of oral, peripheral intravenous, and intraportal glucose on hepatic glucose uptake and insulin and glucagon extraction in conscious dogs. *Journal of Clinical Investigation*, 72, 590–601.

Issekutz, B., Jr (1981). Effects of glucose infusion on hepatic muscle glycogenolysis in exercising dogs. *American Journal of Physiology (Endocrinology and Metabolism)*, 240, E451–E457.

Jackson, L., Williams, F. L. R., Burchell, A., Coughtrie, M. W. H., & Hume, R. (2004). Plasma catecholamines and the counterregulatory responses to hypoglycemia in infants: A critical role for epinephrine and cortisol. *Journal of Clinical Endocrinology and Metabolism*, 89, 6251–6256.

Jackson, P. A., Pagliassotti, M. J., Shiota, M., Neal, D. W., Cardin, S., & Cherrington, A. D. (1997). Effects of vagal blockade on the counterregulatory response to insulin-induced hypoglycemia in the dog. *American Journal of Physiology*, 273, E1178–E1188.

Jacquel, A., Luciano, F., Guillome, R., & Aubgerer, P. (2018). Implication and regulation of AMPK during physiological and pathological myeloid differentiation. *International Journal of Molecular Sciences*, 19(10), 2991. https://doi.org/10.3390/ijms19102991

Janah, L., Kjeldsen, S., Galsgaard, K. D., Winther-Sørensen, M., Stojanovska, E., Pedersen, J., Knop, F. K., & Holst, J. (2020). Weyer Albrechtsen NJ. Glucagon receptor signaling and glucagon resistance. *International Journal of Molecular Sciences*, 20, 3314 (1–27).
Jaspan, J. B., Ruddick, J., & Rayfield, E. (1984). Transhepatic glucagon gradients in man: Evidence for glucagon extraction by human liver. The Journal of Clinical Endocrinology and Metabolism, 58, 287–292.

Jean, C., Tancrède, G., Rousseau-Migneret, S., & Nadeau, A. (1991). Plasma epinephrine in chronic adrenomedullated rats: Lack of response to acute or chronic exercise. Canadian Journal of Physiology and Pharmacology, 69, 1217–1221.

Jefferson, L. S., Exton, J. H., Butcher, R. W., Sutherland, E. W., & Jitrapakdee, S. (2012). Transcription factors and coactivators controlling nutrient and hormonal regulation of hepatic gluconeogenesis. International Journal of Biochemistry & Cell Biology, 44, 33–45. https://doi.org/10.1016/j.biocel.2011.10.001

Jelinek, L. J., Lok, S., Rosenberg, G. B., Smith, R. A., Grant, F., Biggs, S., Bensch, P. A., Kuijper, J. L., Sheppard, P. O., Sprecher, C. A., O’Hara, P. J., Foster, D., Walker, K. M., Chen, L. H. J., McKernan, P. A., & Kindsvogel, W. (1993). Expression cloning and signaling properties of the rat glucagon receptor. Science, 259, 1614–1616. https://doi.org/10.1126/science.8384375

Jiang, G., & Zhang, B. B. (2003). Glucagon and regulation of glucose metabolism. American Journal of Physiology, 284, E671–E678. https://doi.org/10.1152/ajpendo.00492.2002

Jitrapakdee, S. (2012). Transcription factors and coactivators controlling nutrient and hormonal regulation of hepatic gluconeogenesis. International Journal of Biochemistry & Cell Biology, 44, 33–45. https://doi.org/10.1016/j.biocel.2011.10.001

Johannessen, M., & Moens, U. (2007). Multisite phosphorylation of the cAMP response element binding-protein (CREB) by a diversity of protein kinases. Frontiers in Bioscience, 12, 1814–1832. https://doi.org/10.2741/2190

Johnson, D. G., Goebel, C. U., Hruby, V. J., Bregman, M. D., & Trivedi, D. (1982). Hyperglycemia of diabetic rats decreased by a glucagon receptor antagonist. Science, 215, 1115–1116. https://doi.org/10.1126/science.6278587

Joseph, S. K., & Ryan, S. V. (1993). Phosphorylation of the inositol phosphate receptor in isolated rat hepatocytes. Journal of Biological Chemistry, 268, 23059–23065.

Juhl, H., Sheoran, J. H., Schwerer, C. M., Jett, M. F., & Soderling, T. R. (1983). Phosphorylation site specificities of glycogen synthase kinases: Determination by peptide mapping using high-performance liquid chromatography. Archives of Biochemistry and Biophysics, 222, 518–526.

Kaira, S., Unnikrishnan, A. G., Barvah, M. P., Sahay, R., & Bantwal, G. (2021). Metabolic and energy imbalance in dyslycemia-based chronic disease. Diabetes, Metabolic Syndrome and Obesity, 14, 165–184.

Kalkhoff, R. K., Gossain, V. V., & Matute, M. L. (1973). Plasma glucagon in obesity: Response to arginine, glucose, and protein administration. The New England Journal of Medicine, 289, 465–467.

Karlstsson, S., Scheurink, A. K. W., & Ahrén, B. (2002). Gender difference in the glucagon response to glucopenic stress in mice. American Journal of Physiology, 282, R281–R288.

Kass, G. E., Gahm, A., & Lopis, J. (1994). Cyclic AMP stimulates Ca2+ entry in rat hepatocytes by interacting with the plasma membrane carriers involved in receptor-mediated Ca2+ influx. Cellular Signalling, 6, 493–501.

Kavianipour, M., Ehlers, M. R., Malmberg, K., Ronquist, G., Ryden, L., Widström, G., & Gutniak, M. (2003). Glucagon-like peptide-1 (7–36)amide prevents the accumulation of pyruvate and lactate in the ischemic and non-ischemic porcine myocardium. Peptides, 24, 569–578.

Kawai, Y., & Arinze, I. J. (1993). Glucocorticoid regulation of G-protein subunits in neonatal liver. Molecular and Cellular Endocrinology, 90, 203–209. https://doi.org/10.1016/0303-7207(93)90153-B

Keppens, S., Vandekerckhova, A., Moshage, H., Yap, S. H., Aerts, R., & De Wulf, H. (1993). Regulation of glucogen phosphorylase activity in isolated human hepatocytes. Hepatology, 17, 610–614. https://doi.org/10.1002/hep.1840170414

Khan, B. A., Bregman, M. D., Nugent, C. A., Hruby, V. J., & Brendel, K. (1980). (Des-histidineN(N2-phenylthiocarbamoyllysine)s2) - glucagon: Effects on glycogenolysis in perfused rat liver. Biochemical and Biophysical Research Communications, 93, 729–736.

Kinoshita, Y., Nonaka, H., Suzuki, S., Kondo, T., Chihara, K., Chiba, T., Fujita, T., Kotoura, Y., & Yamamura, T. (1985). Accurate localization of insulinoma using percutaneous transhepatic portal venous sampling – usefulness of simultaneous measurement of plasma insulin and glucagon levels. Clinical Endocrinology, 23, 587–593. https://doi.org/10.1111/j.1365-2265.1985.tb0119.x

Klein, C., & Malviya, A. N. (2008). Mechanism of nuclear calcium signaling by inositol-1,4,5-trisphosphate produced in the nucleus, nuclear located protein kinase C and cyclic AMP-dependent protein kinase. Frontiers in Bioscience, 13, 1206–1226.

Kleineke, J., & Söling, H.-D. (1987). The Ca2+/calmodulin-dependent actions of the α-adrenergic agonist phenylephrine on hepatic glycogenolysis differ from those of vasopressin and angiotensin. European Journal of Biochemistry, 112, 143–150.

Knop, F. K., Vilsbøll, T., Madsbad, S., Holst, J. J., & Krarup, T. (2007). Inappropriate suppression of glucagon during OGTT but not during isoglycaemic glucose infusion contributes to the reduced incretin effect in type 2 diabetes. Diabetologia, 50, 797–805.

Kobayashi, M., Sato, H., Matsu, T., Kusunoki, Y., Tokushima, M., Watada, H., Namba, M., & Kitamura, T. (2020). Plasma glucagon levels measured by sandwich ELISA are correlated with impaired glucose tolerance in type 2 diabetes. Endocrine Journal, 67, 903–922. https://doi.org/10.1507/endocrj.E120-0079

Koo, S. H., Flechner, L., Qi, L., Zhang, X., Screakton, R. A., Jeffries, S., Hedrick, S., Xu, W., Boussouar, F., Brindle, P., Takemori, H., & Montminy, M. (2005). The CREB coactivator TORC2 is a key regulator of fasting glucose metabolism. Nature, 437(7062), 1109–1114. https://doi.org/10.1038/nature03967

Kraft, G., Coate, K. C., Winnick, J. J., Dardevet, D., Donahue, E. P., Cherrington, A. D., Williams, P. E., & Moore, M. C. (2017). Glucagon’s effect on liver protein metabolism in vivo. American Journal of Physiology, 313, E263–E272.

Kraus-Friedmann, N. (1984). Hormonal regulation of hepatic gluconeogenesis. Physiological Reviews, 64, 170–259.

Kraus-Friedmann, N. (1986). Effects of glucagon and vasopressin on hepatic Ca2+ release. Proceedings of the National Academy of Sciences, 83, 8943–8946.

Knorre, W., Huttner, W. B., Seitz, H. J., & Tarnowski, W. (1976). Effect of cold exposure on phosphoenolpyruvate carboxykinase (GTP) activity and cyclic AMP concentration in livers of starved rats. Role of Glucocorticoids. Biochim Biophys Acta, 444, 694–703. https://doi.org/10.1016/0005-2760(76)90316-0

Kotzar, A., Bihoreau, M.-T., Nurjhan, N., Picon, L., & Girard, G. (1985). Insulin and glucagon during the perinatal period: Secretion and metabolic effects on the liver. Biology of the Neonate, 48, 204–220.
Langhans, W., Pantel, K., Müller-Shell, W., Eggenberger, E., & Scharrer, E. (1984). Hepatic handling of pancreatic glucagon and glucose during meals in rats. American Journal of Physiology, 247, R827–R832. https://doi.org/10.1152/ajpregu.1984.247.5.R827

Latour, M. G., Bergeron, R., & Lavoie, J.-M. (1999). Effects of hepatic portal infusion of hypertonic saline on glucagon response to exercise. Physiology & Behavior, 67, 377–383. https://doi.org/10.1016/S0031-9384(99)00083-9

Laville, M., Khalifallah, Y., Vidal, H., Beylot, M., Comte, B., & Riou, J. P. (1987). Hormonal control of glucose production and pyruvate kinase activity in isolated rat liver cells: Influence of hypothyroidism. Molecular and Cellular Endocrinology, 50, 247–253.

Lee, Y., Berglund, E. D., Wang, M.-Y., Fu, X., Yu, X., Charron, M. J., Burgess, S. C., & Unger, R. H. (2012). Metabolic manifestations of insulin deficiency do not occur without glucagon action. Proceedings of the National Academy of Sciences of the United States of America, 37, 14972–14976.

Lewis, G. F., Vranic, M., Harley, P., & Giacca, A. (1997). Fatty acids mediate the acute extrahepatic effects of insulin on hepatic glucose production in humans. Diabetes, 46, 1111–1119.

Li, K., Qiu, C., Sun, P., Liu, D.-C., Wu, T.-J., Wang, K., Zhou, Y.-C., Chang, X.-A., Yin, Y., Chen, F., Zhu, Y.-X., & Han, X. (2019). Ets1-mediated acetylation of FoxO1 is critical for gluconeogenesis regulation during feed-fast cycles. Cell Reports, 26, 2998–3010.

Li, X. C., Carretero, O. A., Shao, Y., & Zhuo, J. L. (2006). Glucagon receptor-mediated extracellular signal-related kinase ½ phosphorylation in rat mesangial cells: Role of protein kinase A and phospholipase C. Hypertension, 47, 580–585.

Lin, H. V., & Accili, D. (2011). Hormonal regulation of hepatic glucose production in health and disease. Cell Metabolism, 14, 9–19.

Liu, J., Li, Y., Zhou, X., Zhang, X., Meng, H., Liu, S., Zhang, L., He, J., He, Q., & Geng, Y. (2020). CaMKIV limits metabolic damage through induction of hepatic autophagy by CREB in obese mice. Journal of Endocrinology, 244, 353–367.

Livingston, J. N., Einarsen, K., Backman, L., Ewerth, S., & Amer, P. (1985). Glucagon receptor of human liver. Journal of Clinical Investigation, 75, 397–403.

Lochhead, P. A., Salt, I. P., Walker, K. S., Hardie, D. G., & Sutherland, C. (2000). 5-Aminooimidazole-4-carboxamide ribonucleoside mimics the effects of insulin on the expression of the 2 key gluconeogenic genes PEPCK and glucose-6-phosphatase. Diabetes, 49, 896–903. https://doi.org/10.2337/diabetes.49.6.896

Lochner, A., & Moolman, J. A. (2006). The many faces of H89: A review. Cardiovascular Drug Reviews, 24, 267–274.

Longuet, C., Sinclair, E. M., Maida, A., Baggio, L. L., Maziarz, M., Charron, M. J., & Drucker, D. J. (2008). The glucagon receptor is required for the adaptive response to fasting. Cell Metabolism, 8, 359–371.

Loten, E. G., Assimacopoulos-Jeannet, F. D., Exton, J. H., & Park, C. R. (1978). Stimulation of a low Km phosphodiesterase from liver by insulin and glucagon. Journal of Biological Chemistry, 253, 746–757. https://doi.org/10.1016/S0021-9258(17)38166-8

Lutz, T. A., Estermann, A., Geary, N., & Scharrer, E. (2001). Physiological effect of circulating glucagon on hepatic membrane potential. American Journal of Physiology, 281, R1540–R1544.

Luyckx, A. S., Benedetti, F. M., Falorni, A., & Lefevre, P. J. (1972). Presence of pancreatic glucagon in the portal plasma of human neonates. Differences in the insulin and glucagon responses to glucose between normal infants and infants from diabetic mothers. Diabetologia, 8, 296–300. https://doi.org/10.1007/BF01225575

Lynch, C. J., Blackmore, P. F., Johnson, E. H., Wange, R. L., Krone, P. K., & Exton, J. H. (1989). Guanine nucleotide binding regulator proteins and adenylate cyclase in livers of streptozotocin- and BB/Wor-diabetic rats. Journal of Clinical Investigation, 83, 2050–2062.

Lynch, C. L., Charest, R., Bocckino, S. B., Exton, J. H., & Blackmore, P. F. (1985). Inhibition of hepatic α₁-adrenergic and binding by phorbol myristate acetate. Journal of Biological Chemistry, 260, 2844–2851.

Lyonnet, S., Coupé, C., Girard, G., Kahn, A., & Munnich, R. (1988). In vivo regulation of glycolytic and gluconeogenic gene expression in in newborn rat liver. Journal of Clinical Investigation, 81, 1682–1689.

Manganelli, V., & Vaughn, M. (1972). An effect of dexamethasone on adenosine 3',5'-monophosphate content and adenosine 3',5'-monophosphate phosphodiesterase activity of cultured hepatoma cells. Journal of Clinical Investigation, 51, 2763–2767.

Marcelo, K. L., Means, A. R., & York, B. (2016). The Ca2⁺/calmodulin/CaM/KK2 axis: Nature's metabolic CaMshaft. Trends in Endocrinology and Metabolism, 27, 705–718.

Marks, J. S., & Parker Botelho, L. H. (1986). Synergistic inhibition of glucagon-induced effects on hepatic glucose metabolism in the presence of insulin and a cAMP antagonist. Journal of Biological Chemistry, 261, 15895–15899.

Marllis, E. B., Aoki, T. T., Unber, R. H., Soeldner, J. S., & Cahill, G. F. Jr (1970). Glucagon levels and metabolic effects in fasting man. Journal of Clinical Investigation, 49, 2256–2270.

Marty, N., Dallaporta, M., Foretz, M., Emery, M., Tarussio, D., Bady, I., Binnert, C., Beermann, F., & Thorens, B. (2005). Regulation of glucagon secretion by glucose transporter type to (glut2) and astrocyte-dependent glucose sensors. Journal of Clinical Investigation, 115, 3545–3553.

Mauger, J. P., Claret, M., Petri, F., & Hilly, M. (1989). Hormonal regulation of inositol 1,4,5-triphosphate receptor in rat liver. Journal of Biological Chemistry, 264, 8821–8826.

Mayo, K. E., Miller, L. J., Bataille, D., Dalle, S., Goke, B., Thorens, B., & Drucker, D. J. (2003). International Union of Pharmacology XXXV. The Glucagon Receptor Family. Pharmacol Rev, 55, 167–194.

Mayor, F., & Cuezva, J. M. (1985). Hormonal and metabolic changes in the perinatal period. Biology of the Neonate, 48(48), 185–196. https://doi.org/10.1159/000242171

Mayor, P., & Calle, C. (1988). Glucagon binding and lipolytic response in isolated hepatocytes from streptozotocin-diabetic rats. Endocrinology Japan, 35, 207–215.

McGarry, J. D., & Brown, N. F. (1997). The mitochondrial carnitine palmitoyl transferase system: From concept to molecular analysis. European Journal of Biochemistry, 244, 1–14.

McLeod, M. K., Carlson, D. E., & Gann, D. S. (1983). Secretory response of glucagon to hemorrhage. Journal of Trauma, 23, 445–452.

McNeill, D. A., Herbein, J. H., & Ritchey, S. J. (1982). Hepatic gluconeogenic enzymes, plasma insulin and glucagon response to magnesium deficiency and fasting. Journal of Nutrition, 112, 736–743. https://doi.org/10.1093/jn/112.4.736

Meek, T. H., Dorfman, M. D., Matsen, M. E., Fischer, J. D., Cubelo, A., Kumar, M. R., Taborsky, G. J. Jr, & Morton, G. J. (2015).
Evidence that in uncontrolled diabetes, hyperglucagonemia is required for ketosis but not for increased hepatic glucose production or hyperglycemia. *Diabetes*, 64, 2376–2387.

Mehta, A., Wooten, R., Cheng, K. N., Penfold, P., Halliday, D., & Stacey, T. E. (1987). Effect of diazoxide or glucagon on hepatic glucose production rate during extreme neonatal hypoglycemia. *Archives of Disease in Childhood*, 62, 924–930.

Mihaylova, M. M., & Shaw, R. J. (2011). The AMP-activated protein kinase (AMPK) signaling pathway coordinates cell growth, autophagy, and metabolism. *Nature Cell Biol*, 13, 1016–1023.

Miller, R. A., & Birnbaum, M. J. (2016). Glucagon: Acute actions on hepatic metabolism. *Diabetologia*, 59, 1376–1381.

Miller, R. A., Chu, Q., Xie, J., Foretz, M., Viollet, B., & Birnbaum, M. J. (2013). Biguanides suppress hepatic glucagon signaling by decreasing production of cyclic AMP. *Nature*, 494, 256–260.

Miller, T. B. Jr (1979). Glucose activation of liver glycogen synthase. Insulin-mediated restoration of glucose effect in diabetic rats is blocked by protein synthesis inhibitor. *Biochimica Et Biophysica Acta*, 583, 36–46.

Milner, R. D. G., Fekete, M., Assan, R., & Hodge, J. S. (1972). Effect of glucose on plasma glucagon, growth hormone, and insulin in exchange transfusion. *Archives of Disease in Childhood*, 47, 179–185. https://doi.org/10.1136/adc.47.2.179

Mine, T., Kojima, I., & Ogata, E. (1988). Evidence of cyclic AMP-independent action of glucagon on calcium mobilization in rat hepatocytes. *Biochimica Et Biophysica Acta*, 970, 166–171. https://doi.org/10.1016/0167-4888(88)90175-9

Miura, H., Gardemann, A., Rosa, J., & Jungermann, K. (1992). Inhibition by noradrenaline and adrenaline of the increase in glucose and lactate output and decrease in flow after sympathetic nerve stimulation in perfused rat liver: Possible involvement of protein kinase C. *Hepatology*, 15, 477–484.

Miyauchi, A., Kobayashi, M., Mieno, E., Goto, M., Furusawa, K., Inagaki, T., & Kitamura, T. (2017). Accurate analytical method for human plasma glucagon levels using liquid chromatography-high resolution mass spectrometry: Comparison with commercially available immunoassays. *Analytical and Bioanalytical Chemistry*, 409, 5911–5918.

Milewski, W., Paletta, B., Trupe, W., Paschke, E., & Grimus, R. (1981). Plasma concentrations of glucose, corticosterone, glucagon and insulin and liver content of metabolic substrates and enzymes during starvation and additional hypoxia in the rat. *Hormone and Metabolic Research*, 13, 612–614.

Molinari, D., Angeletti, G., Santesuano, F., & Falorni, A. (1982). Blood glucose, plasma insulin and glucagon response to intravenous administration of glucose in premature infants during the first week of life. *Journal of Endocrinological Investigation*, 5, 169–171. https://doi.org/10.1007/BF00349474

Moore, M. C., Connolly, C. C., & Cherrington, A. D. (1998). Autoregulation of hepatic glucose production. *European Journal of Endocrinology*, 138, 240–248. https://doi.org/10.1530/eje.1.1380240

Moore, M. C., Smith, M. S., Sinha, V. P., Beals, J. M., Michael, M. D., Jakober, S. J., & Cherrington, A. D. (2014). Novel PEGylated basal insulin LY2605541 has a preferential hepatic effect on glucose metabolism. *Diabetes*, 63, 494–504.

Morel, A., O’Carroll, A. M., Brownstein, M. J., & Lolait, S. J. (1992). Molecular cloning of a rat V1a arginine vasopressin receptor. *Nature*, 356, 523–526.

Mortimore, G. E., & Posso, A. R. (1987). Intracellular protein catalysis and its control during nutrient deprivation and supply. *Annual Review of Nutrition*, 7, 539–567.

Movassat, J., Saulnier, C., Serradas, P., & Portha, B. (1997). Impaired development of pancreatic beta-cell mass is a primary event during the progression to diabetes in the GK rat. *Diabetologia*, 40, 916–925.

Müller, M. J., Paschen, U., & Seitz, H. J. (1984). Effect of ketone bodies on glucose production in the miniature pig. *Journal of Clinical Investigation*, 74, 249–261.

Müller, T. D., Finan, B., Clemmensen, C., DiMarchi, R. D., & Tschöp, M. H. (2017). The new biology and pharmacology of glucagon. *Physiological Reviews*, 97, 721–766. https://doi.org/10.1152/physrev.00025.2016

Müller, W. A., Faloona, G. R., & Unger, R. H. (1973). Hyperglucagonemia in diabetic ketoacidosis. Its prevalence and significance. *American Journal of Medicine*, 54, 52–57.

Munday, M. R., Milic, M. R., Takhar, S., Holness, M. J., & Sugden, M. C. (1991). The short-term regulation of hepatic acetyl-CoA carboxylase during starvation and re-feeding in the rat. *The Biochemical Journal*, 280, 733–737.

Mutel, E., Gautier-Stein, A., Abdul-Wahed, A., Amigó-Correig, M., Zitoun, C., Stefanutti, A., Houberson, I., Tourette, J. A., Mittieux, G., & Rajas, F. (2011). Control of blood glucose in the absence of hepatic glucose production during prolonged fasting in mice. *Diabetes*, 60, 3121–3131.

Nair, K. S., Halliday, D., Ford, C., Heels, S., & Garrow, J. S. (1987). Failure of carbohydrate to spare leucine oxidation in obese subjects. *International Journal of Obesity*, 11, 537–544.

Newton, A. C. (2018). Protein kinase C: Perfectly balanced. *Critical Reviews in Biochemistry and Molecular Biology*, 53, 208–230.

Nimmo, H. G., & Cohen, P. (1977). Hormonal control of protein phosphorylation. *Advances in Cyclic Nucleotide Research*, 8, 145–266.

Nishizuka, Y. (1989). Studies and prospectives of the protein kinase C family for cellular regulation. *Cancer*, 63, 1892–1903.

Noguchi, A., Jett, P. A., & Gold, A. H. (1985). cAMP-independent stimulation of glycogen phosphorylase in newborn rat hepatocytes. *American Journal of Physiology (Endocrinology and Metabolism)*, 248, E560–E566.

Nordlie, R. C., Foster, J. D., & Lange, A. J. (1999). Regulation of glucose production by the liver. *Annual Review of Nutrition*, 19, 379–406.

Nurjhan, N., Ktorza, A., Ferre, P., Girard, J. R., & Picon, L. (1985). Effects of gestational hyperglycemia on glucose metabolism and its hormonal control in the fasted, newborn rat during the early postnatal period. *Diabetes*, 34, 995–1001. https://doi.org/10.2337/dbiab.34.10.995

Ogata, E. S., Collins, J. W., & Finley, S. (1988). Insulin injection in the fetal rat: Accelerated intrauterine growth and altered fetal and neonatal glucose homeostasis. *Metabolism*, 37, 649–655.

Ogata, E. S., Paul, R. J., & Finley, S. L. (1987). Limited maternal fuel availability due to hyperinsulinemia retards fetal growth and development in the rat. *Pediatric Research*, 22, 432–437. https://doi.org/10.1203/00006450-198710000-00014

Ogias, D., de Andrade, R., Sá, E., Kasai, A., Moisan, M.-P., Alvare, E. P., & Gama, P. (2010). Fasting differentially regulates plasma corticosterone-binding globulin, glucocorticoid receptor, and cell cycle in the gastric mucosa of pups and adult rats. *American
Oh, K. J., Han, H.-S., Kim, M.-J., & Koo, S.-H. (2013). CREB and FoxO1: Two transcription factors for the regulation of hepatic gluconeogenesis. *BMB Reports*, 46, 567–574.

Ohneda, A., Aguiar-Parada, E., Eisenraut, A. M., & Unger, R. H. (1969). Control of pancreatic glucagon secretion by glucose. *Diabetes*, 18, 1–10.

Okar, D. A., Wu, C., & Lange, A. J. (2004). Regulation of the regulatory enzyme, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase. *Advances in Enzyme Regulation*, 44, 123–154. https://doi.org/10.1016/j.advenzreg.2003.11.006

Okba, A., Hosny, S. S., Elsherbeny, A., & Kamal, M. M. (2020). Study of possible relation between plasma glucagon, gestational diabetes, and development of type 2 diabetes mellitus. *Current Diabetes Reviews*, 16, 148–155.

Okuda, Y., Peña, J., Chou, J., & Field, J. B. (1994). Effect of growth hormone on hepatic glucose and insulin metabolism after oral glucose in conscious dogs. *American Journal of Physiology (Endocrinology and Metabolism)*, 267, E454 - #460.

Omer, A., Duvièrie-Kali, V. F., Aschenbach, W., Chipashvili, V., Goodyear, L. J., & Weir, G. C. (2004). Exercise induces hyperglycemia in rats with islet transplantation. *Diabetes*, 53, 360–365.

Ong, H. L., & Ambudkar, S. (2020). The endoplasmic reticular-plasma membrane junction: A hub for agonist regulation of calcium entry. *Cold Spring Harb Perspect Biol*, 12, a035253. https://doi.org/10.1101/cshperspect.a035253

Opherk, C., Tronche, F., Kellendonk, C., Kohlmüller, C., Schulze, A., Schmid, W., & Schütz, G. (2004). Inactivation of the glucocorticoid receptor in hepatocytes leads to fasting hyperglycemia and ameliorates hyperglycemia in streptozotocin-induced diabetes mellitus. *Molecular Endocrinology*, 18, 1346–1353.

Ozcan, L., Wong, C. C. L., Li, G., Xu, T., Pajvani, U., Park, S. K. R., Wronska, A., Chen, B.-X., Marx, A. R., Fuamizu, A., Backs, J., Singer, H. A., Yates, J. R. III, Accili, D., & Tabas, I. (2012). Calcium signaling through CaMKII regulates hepatic glucose production in fasting and obesity. *Cell Metabolism*, 15, 739–751.

Parker, J. C., Andrews, K. M., Allen, M. R., Stock, J. L., & McNeish, J. D. (2002). Glycemic control in mice with targeted disruption of the glucagon receptor gene. *Biochemical and Biophysical Research Communications*, 290, 839–843.

Patel, D. G. (1984). Role of sympathetic nervous system in glucagon response to insulin hypoglycemia in normal and diabetic rats. *Diabetes*, 33, 1154–1159.

Patil, M., Deshmukh, N. J., Patel, M., & Sangle, G. V. (2020). Glucagon-based therapy: Past, present and future. *Peptides*, 127, 170296. https://doi.org/10.1016/j.peptides.2020.170296

Penke, R. R., James, F. D., Lacy, D. B., Jabbour, K., Williams, P. E., Fueger, P. T., & Wasserman, D. H. (2004). Exercise-induced changes in insulin and glucagon are not required for enhanced glucose uptake after exercise but influence the fate of glucose within the liver. *Diabetes*, 53, 3041–3047.

Pereira, M. J., Thombare, K., Sarsenbayeva, A., Kamble, P. G., Aimbry, K., Lundqvist, M., & Erikkson, J. W. (2020) Direct effects of glucagon on glucose uptake and lipolysis in human adipocytes. *Molecular and Cellular Endocrinology*, 503, 110696. https://doi.org/10.1016/j.mce.2019.110696

Perry, R., Zhang, D., Guerra, M. T., Brill, A. L., Goedeke, L., Nasiri, A. R., Rabin-Court, A., Wang, Y., Peng, L., Dufour, S., Zhang, Y., Zhang, X.-M., Butrico, G. M., Toussaint, K., Nozaki, Y., Cline, G. W., Petersen, K. F., Nathanson, M. H., Ehrlich, B. E., & Shulman, G. I. (2020). Glucagon stimulates gluconeogenesis by INSR31-mediated hepatic lipolysis. *Nature*, 579, 279–283.

Petersen, K. E., & Sullivan, J. T. (2001). Effects of a novel glucagon receptor antagonist (Bay 27–995) on glucagon-stimulated glucose production in humans. *Diabetologia*, 44, 2018–2024.

Pilkis, S. J., Claus, T. H., Johnson, R. A., & Park, C. R. (1975). Hormonal control of cyclic 3′:5′-AMP levels and gluconeogenesis in isolated hepatocytes from fed rats. *Journal of Biological Chemistry*, 250, 6328–6336.

Pilkis, S. J., Exton, J. H., Johnson, R. A., & Park, C. R. (1974). Effects of glucagon on cyclic AMP and carbohydrate metabolism in livers from diabetic rats. *Biochimica Et Biophysica Acta*, 343, 250–267. https://doi.org/10.1016/0304-4165(74)90258-X

Pilkis, S. J., & Ghranner, D. K. (1992). Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annual Review of Physiology*, 54, 884–909. https://doi.org/10.1146/annurev.ph.54.030192.004321

Pingoud, V. A., Peters, F., Haas, T. D. U., & Trautschold, I. (1982). A quantitative analysis of glucagon binding to isolated and intact neonatal and adult rat hepatocytes on the basis of two different binding models. *Biochimica Et Biophysica Acta*, 714, 448–455.

Pittner, R. A., & Spitzer, J. A. (1993). LPS inhibits PI-phospholipase C but not PC-phospholipase D or phosphorylase activation by vasopressin or norepinephrine. *American Journal of Physiology*, 264, E465–E470.

Poggioli, J., Mauger, J. P., & Claret, M. (1986). Effect of cyclic AMP-dependent hormones and Ca2+ mobilizing hormones on the Ca2+ influx and polyphosphoinositide metabolism in isolated rat hepatocytes. *The Biochemical Journal*, 235, 663–669.

Pohl, S. J., Krans, M. J., Birnbaumer, L., & Rodbell, M. (1972). Inactivation of glucagon by plasma membranes of rat liver. *Journal of Biological Chemistry*, 247, 2295–2301.

Porcellati, F., Pampaloni, S., Rossetti, P., Cordoni, C., Marzotti, S., Scionti, L., Bolli, G. B., & Fanelli, C. G. (2003). Counterregulatory hormone and symptomatic responses to insulin-induced hypoglycemia in the postprandial state in humans. *Diabetes*, 52, 2774–2783.

Portha, B., Chamras, H., Broer, Y., Picon, L., & Rosselin, G. (1983). Decreased glucagon-stimulated cyclic AMP production by isolated liver cells of rats with type 2 diabetes. *Molecular and Cellular Endocrinology*, 32, 13–26.

Powell, A. M., Sherwin, R. S., & Shulman, G. I. (1993). Impaired hormonal responses to hypoglycemia in spontaneously diabetic and recurrently hypoglycemic rats. *Journal of Clinical Investigation*, 92, 2667–2674.

Puri, B. K. (2020). Calcium signaling and gene expression. *Advances in Experimental Medicine and Biology*, 1131, 537–545. https://doi.org/10.1007/978-3-030-12457-1_22

Püscher, G. P., Miura, H., Neuschäler-Rube, F., & Jungermann, K. (1993). Inhibition by the protein kinase C activator 4β-phorbol 12-myristate 13-acetate of the prostaglandin F2alpha-mediated and noradrenaline-mediated but not glucagon-mediated activation of glycogenolysis in rat liver. *European Journal of Biochemistry*, 217, 305–311.

Rabouti, N., Arem, R., Jones, R. H., Chap, Z., Pena, J., Chou, J., & Field, J. B. (1989). Fasting and postabsorptive hepatic glucose and insulin metabolism in hyperthyroidism. *American Journal of Physiology (Endocrinology and Metabolism)*, 253, E159–E166.
Racioppi, L., & Means, A. R. (2012). Calcium/calmodulin dependent protein kinase kinase 2: Roles in signaling and pathophysiology. Journal of Biological Chemistry, 287, 31658–31665.

Raju, B., & Cryer, P. E. (2005). Maintenance of the postabsorptive plasma glucose concentration: insulin or insulin plus glucagon? American Journal of Physiology, 289, E181–E186. https://doi.org/10.1152/ajpendo.00460.2004

Rao, R. H. (1995). Fasting glucose homeostasis in the adaptation to chronic nutritional deprivation in rats. American Journal of Physiology (Endocrinology and Metabolism), 268, E873–E879. https://doi.org/10.1152/ajpendo.1995.268.5.E873

Ravier, M. A., & Rutter, G. A. (2005). Glucose or insulin, but not zinc ions, inhibit glucagon secretion from mouse pancreatic alpha-cells. Diabetes, 54, 1789–1797.

Ravnskjaer, K., Madiraju, A., & Montminy, M. (2015). Role of the cAMP pathway in glucose and lipid metabolism. In S. Herzg (Ed.), Metabolic control. Handbook of experimental pharmacology, Vol. 233 (pp. 29–49). Springer International. https://doi.org/10.1007/164_2015_32

Reber, B. F. X., Somogyi, R., & Stucki, J. W. (1990). Hormone-induced intracellular calcium oscillations and mitochondrial energy supply in single hepatocytes. Biochimica Et Biophysica Acta, 1018, 190–193. https://doi.org/10.1016/0005-2728(90)90246-Z

Rider, M. H., Bertrand, L., Vertommen, D., Michels, P. A., Rousseau, G. G., & Hue, L. (2004). 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase: head-to-head with a bifunctional enzyme that controls glycolysis. The Biochemical Journal, 231, 561–579.

Rivera, N., Ramnanan, C. J., An, Z., Farmer, T., Smith, M., Farmer, B., Irinima, J. M., Snead, W., Lautz, M., Roach, P. J., & Cherrington, A. D. (2010). Insulin-induced hypoglycemia increases hepatic sensitivity to glucagon in dogs. Journal of Clinical Investigation, 120, 4425–4435. https://doi.org/10.1172/JCI40919

Rix, I., Nexo-Elsen, C., Bergmann, N. C., Lund, A., & Knop, F. K. (2019). Glucagon physiology. Endotext [Internet]. https://www.ncbi.nlm.nih.gov/books/NBK279127/

Robberecht, P., Damien, C., Moroder, L., Coy, D. H., Wünsch, E., & Christophe, J. (1988). Receptor occupancy and adenylate cyclase activation in rat liver and heart membranes by 10 glucagon analogs modified in position 2, 3, 4, 25, 27, and/or 29. Regul Peptides, 21, 117–128. https://doi.org/10.1016/0167-0115(88)90096-1

Rodbell, M., Krans, H. M. J., Pohl, S. L., & Birnbaumer, L. (1971). The glucagon-sensitive adenylate cyclase system in plasma membranes of rat liver. III. Binding of glucagon: Method of assay and specificity. Journal of Biological Chemistry, 246, 1861–1871.

Rodgers, R. L. (2012). Glucagon and cyclic AMP: Time to turn the page? Current Diabetes Reviews, 8, 362–381.

Rodgers, R. L. (2021). The hepatic glucose-mobilizing effect of glucagon is not mediated by cyclic AMP most of the time. American Journal of Physiology. Endocrinology and Metabolism, 321, E575–E578.

Roskoski, R. Jr (2015). A historical overview of protein kinases and their targeted small molecule inhibitors. Pharmacological Research, 100, 1–23.

Rothermel, J. D., Jastor, B., & Parker Botelho, L. H. (1984). Inhibition of glucagon-induced glycogenolysis in isolated rat hepatocytes by the Rp diastereomer of adenosine cyclic 3’,5’-phosphorothioate. Journal of Biological Chemistry, 259, 8151–8155.

Rothermel, J. D., Perillo, N. L., Marks, J. S., & Botelho, L. H. (1984). Effects of the specific cAMP antagonist, (Rp)-adenosine cyclic 3’,5’-phosphorothioate, on the cAMP-dependent protein kinase-induced activity of hepatic glycogen phosphorylase and glycogen synthase. Journal of Biological Chemistry, 259, 15294–15300.

Rui, L. (2014). Energy metabolism in the liver. Comprehensive Physiology, 4, 177–197.

Ruiter, M., LaFleur, S. E., van Heijningen, C., van der Vliet, J., Kalsbeek, A., & Buijs, R. M. (2003). The daily rhythm of plasma glucagon concentrations in the rat is modulated by the biological clock and by feeding behavior. Diabetes, 52, 1709–1715.

Saccà, L., Sherwin, R., & Felig, P. (1979). Influence of somatostatin on glucagon- and epinephrine-stimulated hepatic glucose output in the dog. American Journal of Physiology (Endocrinology and Metabolism Gastrointestinal Physiology), 236, E113–E117.

Salle, B. L., & Ruitton-Ugliengo, A. (1977). Effects of oral glucose and protein load on plasma glucagon and insulin concentrations in small for gestational age infants. Pediatric Research, 11, 108–112. https://doi.org/10.1203/00006450-197702000-00005

Salminen, A., Kaarniranta, K., & Kauppinen, A. (2016). Age-related changes in AMPK activation: Role for AMPK phosphatases and inhibitory phosphorylation by upstream signaling pathways. Ageing Research Reviews, 28, 15–26.

Savage, A., Zeng, L., & Houslay, M. D. (1995). A role for protein kinase C-mediated phosphorylation in eliciting glucagon desensitization in rat hepatocytes. The Biochemical Journal, 307, 281–285. https://doi.org/10.1042/bj03070281

Schaub, J., Gutmann, I., & Lippert, H. (1972). Developmental changes of glycolytic and gluconeogenic enzymes in fetal and neonatal rat liver. Hormone and Metabolic Research, 4, 110–119. https://doi.org/10.1055/s-0028-1094080

Schwart, M. W., Strack, A. M., & Dallman, M. F. (1997). Evidence that elevated plasma corticosterone levels are the cause of reduced hypothalamic corticotrophin-releasing hormone gene expression in diabetes. Regulatory Peptides, 72, 105–112. https://doi.org/10.1016/S0167-0115(97)01043-4

Seitz, H. J., Kaiser, M., Krone, W., & Tornowski, W. (1976). Physiological significance of glucocorticoids and insulin in the regulation of hepatic gluconeogenesis during starvation in rats. Metabolism, 25, 1545–1555.

Seitz, H. J., Krone, W., & Tornowski, W. (1977). Physiological regulation of rat liver phosphoenolpyruvate carboxykinase (GTP) by insulin. Significance of a cyclic AMP-independent mechanism. Acta Endocrinol, 85, 389–397. https://doi.org/10.1530/acta.0.0850389

Sellers, T. L., Jaussi, A. W., Yang, H. T., Heninger, R. W., & Winder, W. W. (1988). Effect of the exercise-induced increase in glucocorticoids on endurance in the rat. Journal of Applied Physiology, 65, 173–178.

Semakova, J., Hyraššová, P., Mendez-Lucas, A., Cutz, E., Bermudez, J., Burgess, S., Alcántara, S., & Perales, J. C. (2017). PEPCK-C reexpression counters neonatal hypoglycemia in Pck1del/del mice, unmasking role in non-glucogenic tissues. Journal of Physiology and Biochemistry, 73, 89–98.

Servillo, G., Della Fazia, M. A., & Sassoni-Corsi, P. (2002). Coupling cAMP signaling to transcription in the liver: Pivotal role of CREB and CREM. Experimental Cell Research, 275, 143–154.

Sharabi, K., Tavares, C. D., & Puigserver, P. (2019). Regulation of hepatic metabolism, recent advances, and future perspectives. Current Diabetes Reports, 19, 98. https://doi.org/10.1007/s11892-019-1224-4
Shaywitz, A. J., & Greenberg, M. E. (1999). CREB: A stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annual Review of Biochemistry, 68*, 821–861.

Sherck, S. M., Shiota, M., Saccamondo, J., Cardin, S., Allen, E. J., Hastings, J. R., Neal, D. W., Williams, P. E., & Cherrington, A. D. (2001). Pancreatic response to mild non-insulin induced hypoglycemia does not involve extrinsic neural input. *Diabetes, 50*, 2487–2496. [https://doi.org/10.2337/diabetes.50.11.2487](https://doi.org/10.2337/diabetes.50.11.2487)

Sherwin, R. S., Hendler, R., DeFronzo, R., Wahren, J., & Felig, P. (1977). Glucose homeostasis during prolonged suppression of glucagon and insulin secretion by somatostatin. *Proceedings of the National Academy of Sciences, 74*, 348–352. [https://doi.org/10.1073/pnas.74.1.348](https://doi.org/10.1073/pnas.74.1.348)

Shi, Z. Q., Rastogi, K. S., Lekas, M., Efendic, S., Drucker, D. J., & Vranic, M. (1996). Glucagon response to hypoglycemia is improved by insulin-dependent restoration of of normoglycemia in diabetic rats. *Endocrinology, 137*, 3193–3199.

Shiota, M., Green, R., Colburn, C. A., Mitchell, G., & Cherrington, A. D. (1996). Inability of hyperglycemia to counter the ability of glucagon to increase net glucose output and activate glucagon phospholipase in the perfused rat liver. *Metabolism, 45*, 481–485. [https://doi.org/10.1016/S0026-0495(96)00223-1](https://doi.org/10.1016/S0026-0495(96)00223-1)

Silva, G., Navasa, M., Bosch, J., Chester, J., Pizcueta, M. P., Caramitjana, R., Riverà, F., & Rodès, J. (1990). Hemodynamic effects of glucagon in portal hypertension. *Hepatology, 11*, 668–673. [https://doi.org/10.1002/hep.1840110421](https://doi.org/10.1002/hep.1840110421)

Sim, A. T. R., & Hardie, D. G. (1988). The low activity of acetyl-CoA carboxylase in basal and glucagon-stimulated hepatocytes is due to phosphorylation by the AMP-activated protein kinase and not cyclic AMP-dependent protein kinase. *FEBS Letters, 233*, 294–298.

Sistare, F. D., Picking, R. A., & Haynes, R. C. Jr (1985). Sensitivity of the response of cytosolic calcium in Quin-2-loaded rat hepatocytes to glucagon, adenine nucleosides, and adenosine nucleotides. *Journal of Biological Chemistry, 260*, 12744–12747. [https://doi.org/10.1001/S0021-9258(17)38939-1](https://doi.org/10.1001/S0021-9258(17)38939-1)

Slotkin, T. A., Lorbier, B. A., McCook, E. C., Barnes, G. A., & Seidler, F. J. (1995). Neural input and the development of adrenergic intracellular signaling: Neonatal denervation evokes neither receptor upregulation nor persistent supersensitivity of adenylate cyclase. *Developmental Brain Research, 88*, 17–29.

Slotkin, T. A., Thai, L., McCook, E. C., Saleh, J. L., Zhang, J., & Seidler, F. J. (1996). Aging and glucocorticoids: Effects on cell signaling mediated by adenylate cyclase. *Journal of Pharmacology and Experimental Therapeutics, 278*, 476–491.

Smadja, C., Morin, J., Ferré, P., & Girard, J. (1990). Initial glucose kinetics and hormonal response to a gastric glucose load in unrestrained post-absorptive and starved rats. *The Biochemical Journal, 270*, 505–510.

Soderling, T. R. (1999). The Ca2+ calmodulin dependent protein kinase cascade. *Trends in Biochemical Sciences, 24*, 232–236.

Soman, V., & Felig, P. (1978). Glucagon binding and adenylate cyclase activity in liver membranes from untreated and insulin-treated diabetic rats. *Journal of Clinical Investigation, 61*, 552–560.

Somogyi, R., Zhao, M., & Stucki, J. W. (1992). Modulation of cytosolic-[Ca2+] oscillations in hepatocytes results from crosstalk among second messengers. *The Biochemical Journal, 286*, 869–877.

Sonne, O., Berg, T., & Christoffersen, T. (1978). Binding of 125I-labeled glucagon and glucagon-stimulated accumulation of adenosine 3’:5’-monophosphate in isolated intact rat hepatocytes. *Journal of Biological Chemistry, 253*, 3203–3210.

Soybel, D., Jaspán, J., Polonsky, K., Goldberg, I., Rayfield, E., & Tager, H. (1983). Differential immunoreactivity of plasma glucagon components in man: Studies with different glucagon antibodies. *Journal of Clinical Endocrinology and Metabolism, 56*, 612–618.

Sperling, M. A., DeLamater, P. V., Phelps, D., Fiser, R. H., Oh, W., & Fisher, D. A. (1974). Spontaneous and amino acid-stimulated glucagon secretion in the immediate postnatal period. *Journal of Clinical Investigation, 53*, 1159–1166.

Srikant, C. B., Freeman, D., McKorkle, K., & Unger, R. G. (1977). Binding and biological activity of glucagon in liver cell membranes of chronically hyperglucagonemic rats. *Journal of Biological Chemistry, 252*, 7434–7436.

Staddon, J. M., & Hansford, G. H. (1989). Evidence indicating that the glucagon-induced increase in cytoplasmic free Ca2+ concentration in hepatocytes is mediated by an increase in cyclic AMP concentration. *European Journal of Biochemistry, 179*, 47–52. [https://doi.org/10.1111/j.1432-1033.1989.tb4519.x](https://doi.org/10.1111/j.1432-1033.1989.tb4519.x)

Stanley, C. A., Rozance, P. J., Thornton, P. S., De Leon, D. D., Harris, D., Hammond, M. W., Hussain, K., Levitsky, L. L., Murad, M. H., Simmons, R. A., Sperling, M. A., Weinstein, D. A., White, N. H., & Wolsford, J. I. (2015). Re-evaluating transitional “neonatal hypoglycemia”: Mechanism and implications for management. *Journal of Pediatrics, 166*, 1520–5. [https://doi.org/10.1016/j.jpeds.2015.02.045](https://doi.org/10.1016/j.jpeds.2015.02.045)

Studer, R. K., Snowdowme, K. W., & Borle, A. B. (1984). Regulation of hepatic glycogenolysis by glucagon in male and female rats. *Journal of Biological Chemistry, 259*, 3596–3604.

Sugden, M. C., Howard, R. M., Munday, M. R., & Holness, M. J. (1993). Mechanisms involved in the coordinate regulation of strategic enzymes of glucose metabolism. *Advances in Enzyme Regulation, 33*, 71–95. [https://doi.org/10.1016/0065-2571(93)90010-B](https://doi.org/10.1016/0065-2571(93)90010-B)

Sumi, S., Mineo, I., Kono, N., Shimizu, T., Nonaka, K., & Tarui, S. (1984). Decreases in hepatic fructose-2,6-bisphosphate level and fructose-bis-phosphate, 2 kinase activity in diabetic mice: A close relationship to the development of ketosis. *Biochemical and Biophysical Research Communications, 120*, 103–108.

Sun, P., Enslen, H., Myung, P. S., & Maurer, R. A. (1994). Differential activation of CREB by Ca2+/calmodulin-dependent protein kinases type II and type IV involves phosphorylation of a site that negatively regulates activity. *Genes & Development, 8*, 2527–2539.

Sunehag, A., Gustaffson, J., & Ewald, U. (1994). Very immature infants (≤30 wk) respond to glucose infusion with incomplete suppression of glucose production. *Pediatric Research, 36*, 550–555.

Sutherland, E. (1972). Studies on the mechanism of hormone action. *Science, 177*, 401–408.

Taborsky, G. J. (2010). The physiology of glucagon. *Journal of Diabetes Science and Technology, 4*, 1338–1344. [https://doi.org/10.1177/193229681000400607](https://doi.org/10.1177/193229681000400607)
Takekoto-Kimura, S., Suzuki, K., Horigane, S.-I., Kamijo, J., Inoue, M., Sakamoto, M., Fujii, H., & Bito, H. (2017). Calmodulin kinases: Essential regulators in health and disease. *Neurochem, 141*, 808–818.

Tasaka, Y., Inoue, S., Maruno, K., & Hirata, Y. (1980). Twenty-four-hour variations of plasma pancreatic polypeptide, insulin, and glucagon in normal subjects. *Endocrinol Japan, 27*, 495–498.

Taskén, K., & Aandahl, E. M. (2003). Localized effects of cAMP mediated by distinct routes of protein kinase A. *Physiological Reviews, 84*, 137–167.

Thomas, A. P., Renard, D. C., & Rooney, T. A. (1991). Spacial and temporal organization of calcium signaling in hepatocytes. *Cell Calcium, 12*, 111–126.

Trebrien, R., Klarskov, L., Olesen, M., Holst, J. J., Carr, R. D., & Deacon, C. F. (2004). Neutral endopeptidase 24.11 is important for the degradation of both endogenous and exogenous glucan in anesthetize pigs. *American Journal of Physiology, 287*, E431–E438.

Ubl, J., Chen, S., & Stucki, J. W. (1994). Anti-diabetic biguanides inhibit hormone-induced intracellular calcium concentration oscillations in rat hepatocyte. *The Biochemical Journal, 304*, 561–567.

Unger, R. H. (1985). Glucagon physiology and pathophysiology in the light of new advances. *Diabetologia, 28*, 574–578.

Unger, R. H., & Cherrington, A. D. (2012). Glucagononcentric restructuring of diabetes: A pathophyslogic and therapeutic overview. *Journal of Clinical Investigation, 122*, 4–12.

Unson, C. G., Gurzenda, E. M., & Merrifield, R. B. (1989). Biological activities of des-His[1][Glu1] glucagon amide, a glucagon antagonist. *Peptides, 10*, 1171–1177.

Unterman, T. G. (2018). Regulation of hepatic glucose metabolism by FoxO proteins, an integrated approach. *Current Topics in Developmental Biology, 127*, 119–147. https://doi.org/10.1016/bs.ctdb.2017.10.005

Uvnäs-Moberg, K., Posloncec, B., & Åhlberg, L. (1986). Influence on plasma levels of somatostatin, gastrin, glucagon, insulin and VIP-like immunoreactivity in peripheral venous blood of anaesthetized cats induced by low intensity afferent stimulation of the sciatic nerve. *Acta Physiologica Scandinavica, 126*, 225–230.

Vaitkus, P., Sirek, A., Norwich, K. H., Sirek, O. V., Unger, R. H., & Harris, V. (1984). Rapid changes in hepatic glucose output after a pulse of growth hormone in dogs. *American Journal of Physiology, 246*, E14–E20. https://doi.org/10.1152/ajpendo.1984.246.1.E14

van de Wall, E. H. E. M., Gram, D. X., Strubbe, J. H., Scheurink, J. W., & Kooolhaas, J. M. (2005). Ablation of capsacin-sensitive afferent nerves affects insulin response during an intravenous glucose tolerance test. *Life Sciences, 77*, 1283–1292.

Vénien-Bryan, C., Lowe, E. M., Boisset, N., Traxler, K. W., Johnson, L. N., & Carlson, G. M. (2002). Three-dimensional structure of phosphorylase kinase at 22 Å resolution and its complex with glycogen phosphorylase b. *Structure, 10*, 33–41. https://doi.org/10.1016/S0969-2126(01)00691-8

Verrillo, A., de Teresa, A., Martino, C., di Chiara, G., & Verrillo, L. (1988). Somatostatin response to glucose before and after prolonged fasting in lean and obese non-diabetic subjects. *Regul Peptides, 21*, 185–195. https://doi.org/10.1016/0167-0115(88)90001-8

Viana, A. Y. I., Sakoda, H., Anai, M., Fujiyoshi, M., Ono, H., Kushiyama, A., Fukushima, Y., Sato, Y., Oshida, Y., Uchijima, Y., Kurihara, H., & Asano, T. (2006). Role of hepatic AMPK activation in glucose metabolism and dexamethasone-induced AMPK expression. *Diabetes Research and Clinical Practice, 73*, 135–142.

Vinicer, F., Higdon, G., Clark, J. F., & Clark, C. M. Jr (1976). Development of glucagon sensitivity in neonatal rat liver. *Journal of Clinical Investigation, 58*, 571–578.

Violett, B., Guigas, B., Leclerc, J., Hébrard, S., Lantier, L., Mounier, R., Andreelli, F., & Foretz, M. (2009). AMP-activated protein kinase in the regulation of hepatic energy metabolism: From physiology to therapeutic perspectives. *Acta Psychologica, 196*, 81–98.

Wakelam, M. J. O., Murphy, G. J., Hruby, V. J., & Houslay, M. D. (1986). Activation of two signal-transduction systems in hepatocytes by glucagon. *Nature, 323*, 68–70.

Walsh, M. F., & Dunbar, J. C. (1984). Glucagon binding to liver membranes of Mt-W-15 tumor-bearing and hyphophysectomized rats. A possible role for insulin and growth hormone. *Diabetes, 33*, 978–983.

Wang, Y., Camici, M., Lee, F. T., Ahmad, Z., DePaoli-Roach, A. A., & Roach, P. J. (1986). Multiple phosphorylation sites of rat liver glycogen synthase. *Biochimica Et Biophysica Acta, 888*, 225–236.

Wang, Y., Li, G., Goode, J., Paz, J. C., Ouyang, K., Sreeton, R., Fischer, W. H., Chen, J., Tabas, I., & Montminy, M. (2012). InsP3 receptor regulates hepatic glucogenogenesis in fasting and diabetes. *Nature, 485*, 128–132.

Wang, Y., Vera, L., Fischer, W. H., & Montminy, M. (2009). The CREB coactivator CRTC2 links hepatic ER stress and fasting glucogenogenesis. *Nature, 460*, 534–537.

Warner, S. O., Yao, M. V., Cason, R. L., & Winnick, J. J. (2020). Exercise-induced improvements in to whole-body glucose metabolism in type 2 diabetes: The essential role of the liver. *Front Endocrinol (Lausanne), 11*, 567–569.

Wasserman, D. H., Lacy, D. B., & Bracy, D. P. (1993). Relationship between arterial and portal vein immunoreactive glucagon during exercise. *Journal of Applied Physiology, 75*, 724–729.

Watanabe, T., Morimoto, A., Sakata, Y., Wada, M., & Murakami, N. (1991). The effect of chronic exercise on the pituitary-adrenocortical response in conscious rats. *Journal of Physiology, 439*, 691–699.

Wayman, G. A., Todumitsu, H., Davare, M. A., & Soderling, T. R. (2011). Analysis of CaM-kinase signaling in cells. *Cell Calcium, 50*, 1–8.

Wendt, A., & Eliaassi, L. (2020). Pancreaticα-cells: The unsung heroes in islet function. *Seminars in Cell and Developmental Biology, 103*, 21–50. https://doi.org/10.1016/j.semcdb.2020.01.006

Wernette Hammond, M. E., & Lardy, H. A. (1985). Regulation of glucogenesisis in hepatocytes from fasted alloxan-diabetic rats. *Diabetes, 34*, 767–773.

Wewer Albrechtsen, N., Bak, M. J., Hartmann, B., Christensen, L. W., Kuhre, R. E., Deacon, C. F., & Holst, J. G. (2015). Stability of glucagon-like peptide 1 and glucagon in human plasma. *Endocrine Connect, 4*, 50–57.

Wewer Albrechtsen, N. J., Hartmann, B., Veedsfald, S., Windeløv, J. A., Plamboeck, A., Bojsen-Møller, K. N., Idorn, T., Feldt-Rasmussen, T., Knop, F. K., Vilsbøll, T., Madsbad, S., Deacon, C. F., & Holst, J. J. (2014). Hyperglucagonaemia analysed by
glucagon sandwich ELISA: Nonspecific interference or truly elevated levels? *Diabetologia*, 57, 1919–1926.

Wewer Albrechtsen, N. J., Kuhre, R. E., Pedersen, J., Knop, F. P., & Holst, J. J. (2016). The biology of glucagon and the consequences of hyperglucagonemia. *Biomarkers in Medicine*, 10, 1141–1151. https://doi.org/10.2217/bmm-2016-0090

Wewer Albrechtsen, N. J., Kuhre, R. E., Windelov, J. A., Ørgaard, A., Deacon, C. F., Kissow, H., Hartmann, B., & Holst, J. J. (2016). Dynamics of glucagon secretion in mice using a validated sandwich ELISA for small sample volumes. *American Journal of Physiology*, 311, E302–E309.

Wewer Albrechtsen, N. J., Pedersen, J., Galsgaard, K. D., Winther-Sørensen, M., Suppli, M. P., Janah, L., Gromada, J., Vilstrup, H., Knop, F. P., & Holst, J. J. (2019). The liver-α-cell axis and type 2 diabetes. *Endocrine Reviews*, 40, 1353–1366.

Wewer Albrechtsen, N. J., Veedelfd, S., Plamboeck, A., Deacon, C. F., Hartmann, B., Knop, F. K., Vilsboll, T., & Holst, J. J. (2016). Inability of some commercial assays to measure suppression of glucagon secretion. *Journal of Diabetes Research*, 2016, 8352957. https://doi.org/10.1155/2016/8352957

Whipps, D. E., Armstrong, A. E., Pryor, H. J., & Halestrap, A. P. (1987). Effects of glucagon and Ca2+ on the metabolism of phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate in isolated rat hepatocytes and plasma membranes. *The Biochemical Journal*, 241, 835–845.

Wicks, W. D. (1971). Regulation of hepatic enzyme synthesis by cyclic AMP. *Annals of the New York Academy of Sciences*, 185, 152–165.

Widmaier, E. P., Shah, P. R., & Lee, G. (1991). Interactions between oxytocin, glucagon and glucose in normal and streptozotocin-induced diabetic rats. *Regul Peptides*, 34, 235–249.

Williams, E. L., Rodriguez, S. M., Beitz, D. C., & Donkin, S. S. (2006). Effects of short-term glucagon administration on gluconeogenic enzymes in the liver of midlactation dairy cows. *Journal of Dairy Science*, 89, 693–703.

Williamson, J. R., Hansen, C. A., Verhoeven, A., Coll, K. E., Johanson, R., Williamson, M. T., & Filburn, C. (1987). Mechanisms involved in receptor-mediated changes of intracellular calcium in liver. *Society of General Physiologists Series*, 42, 93–116.

Willis, B. S., Niswender, C. M., Su, T., Amieux, T. S., & McKnight, G. S. (2011). Cell-type specific expression of a dominant negative PKA mutation in mice. *PloS One*, 6, e18772. https://doi.org/10.1371/journal.pone.0018772

Willows, R., Sanders, M. J., Xiao, B., Patel, B. K., Martin, S. R., Read, J., Wilson, J. R., Hubbard, J., Gamblin, S. J., & Carling, D. (2017). Phosphorylation of AMPK by upstream kinases is required for activity in mammalian cells. *The Biochemical Journal*, 474, 3059–3073.

Winder, W. W. (1985). Control of hepatic glucose production during exercise. *Medicine & Science in Sports & Exercise*, 17, 2–5. https://doi.org/10.1249/00005768-198502000-00002

Winder, W. W. (1988). Role of cyclic AMP in regulation of hepatic glucose production during exercise. *Medicine & Science in Sports & Exercise*, 20, 551–559. https://doi.org/10.1249/00005768-198812000-00006

Winder, W. W., Arogyasami, J., Yang, H. T., Thompson, K. G., Nelson, L. A., Kelly, K. P., & Han, D. H. (1988). Effects of glucose infusion in exercising rats. *Journal of Applied Physiology*, 64, 2300–2305. https://doi.org/10.1152/jappl.1988.64.6.2300

Winder, W. W., Beattie, M. A., & Holman, R. T. (1982). Endurance training attenuates stress hormone responses to exercise in fasted rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 243(1), R179–R184. https://doi.org/10.1152/ajpregu.1982.243.1.R179

Winder, W. W., Bouliller, J., & Fell, R. D. (1979). Liver glycogenolysis during exercise without a significant increase in cyclic AMP. *American Journal of Physiology*, 237, R147–R152.

Winder, W. W., Holman, R. T., & Garhart, S. J. (1981). Effect of endurance training on liver CAMP response to prolonged submaximal exercise. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology*, 240, R330–R334.

Winder, W. W., Yang, H. T., & Arogyasami, J. (1988). Liver fructose 2,6-bisphosphate in rats running at different treadmill speeds. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology*, 255(1), R38–R41.

Winnzell, M. S., Brand, C. L., Wierup, N., Sidellmann, U. G., Sundler, F., Nixhimura, E., & Ahren, B. (2007). Glucagon receptor antagonism improves islet function in mice with insulin resistance by a high-fat diet. *Diabetologia*, 50, 1453–1462.

Witters, L. A., Kemp, B. E., & Means, A. R. (2006). Chutes and ladders: The search for protein kinases that act on AMPK. *Trends in Biochemical Sciences*, 31, 13–16.

Wolf, E., & Eisenstein, A. B. (1981). Portal vein blood insulin and glucagon are increased in experimental hyperthyroidism. *Endocrinol*, 108, 2109–2133.

Wolle, B. M., Culebras, J. M., Sim, A. J. W., Ball, M. R., & Moore, F. D. (1977). Substrate interaction in intravenous feeding. *Annals of Surgery*, 186, 518–640.

Woods, N. M., Cuthbertson, K. S. R., & Cobbold, P. H. (1986). Repetitive transient rises in cytoplasmic free calcium in hormone-stimulated hepatocytes. *Nature*, 319, 600–602.

Wright, D. C., Craig, B. W., Fick, C. A., & Lim, K. I. (2002). The effects of phospholipase C inhibition on insulin-stimulated glucose transport in skeletal muscle. *Metabolism - Clinical and Experimental*, 51, 271–273.

Wu-Zhang, A. X., & Newton, A. C. (2013). Protein kinase C pharmacology: refining the toolbox. *Biochemical Journal*, 452, 195–209. https://doi.org/10.1042/BJ20130220

Xing, Y.-Q., Li, A., & Yang, Y. (2018). Li X-q, Zhang L-n, Guo H-c. The regulation of Fox01 and its role in disease progression. *Life Sciences*, 193, 124–131.

Xu, Y., & Xie, X. (2009). Glucagon receptor mediates calcium signaling by coupling to Ga<sub>q</sub>11 and Ga<sub>q</sub>12 in HEK293 cells. *Journal of Receptors and Signal Transduction*, 29(6), 318–325.

Xue, N., Cei, C., Zhang, L., Liu, H., Wang, X., & Wang, L. (2017). The characteristics of hepatic Gsa-cAMP axis in HSHF diet-fed obese insulin resistance rats and genetic diabetic mice. *Biological &/and Pharmaceutical Bulletin*, 40, 774–781. https://doi.org/10.1248/bpb.b17-00749

Xue, N., Wei, C., Zhang, L., Liu, H., Wang, X., & Wang, L. (2017). The characteristics of hepatic Gsa-cAMP axis in HSHF diet-fed obese insulin-resistant rats and genetic diabetic mice. *Biological &/and Pharmaceutical Bulletin*, 40, 774–781.

Yabaluri, N., & Bashyam, M. D. (2010). Hormonal regulation of gluconeogenic gene transcription in the liver. *Journal of Biosciences*, 35, 473–484.

Yagami, T. (1995). Differential coupling of glucagon and β-adrenergic receptors with the small and large forms of the stimulatory G protein. *Molecular Pharmacology*, 48, 849–854.
Yamashita, K., Yamashita, S., Yasuda, H., Oka, Y., & Ogata, E. (1980). A decreased response of cyclic adenosine monophosphate concentrations to glucagon in liver slices of streptozotocin-induced diabetic rats. *Diabetes*, 29, 188–192.

Yamatani, K., Saito, K., Ikezawa, Y., Ohnuma, H., Sugiyama, K., Manaka, H., Takahashi, K., & Sasaki, H. (1998). Relative contribution of Ca\(^{2+}\)-dependent mechanism in glucagon-induced glucose output from the liver. *Archives of Biochemistry and Biophysics*, 355, 175–180.

Yamatani, K, Sato, N, Wada, K, Suda, K, Wakasugi, K, Ogawa, A, Takahashi, K, Sasaki, H, & Har, M. (1987). Two types of hormone-responsive adenylate cyclase in the rat liver. *Biochimica Et Biophysica Acta*, 931, 180–187.

Yang, H., & Yang, L. (2016). Targeting cAMP/PKA pathway for glycemic control and type 2 diabetes therapy. *Molecular Endocrinology*, 57, R93–R108.

Yoon, J. C., Pulgserver, P., Chen, G., Donovan, J., Wu, Z., Rhee, J., Adelmant, G., Stafford, J., Kahn, C. R., Granner, D. K., Newgard, C. B., & Spiegelman, B. M. (2001). Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature*, 413, 131–138.

Yoshida, H., Ishii, M., & Akagawa, M. (2019). Propionate suppresses hepatic gluconeogenesis via GPR43/AMPK signaling pathway. *Archives of Biochemistry and Biophysics*, 672, 108057.

Zhang, H., Nielsen, A. L., & Strømgard, K. (2020). Recent achievement in developing selective Gq inhibitors. *Medicinal Research Reviews*, 40, 135–157.

Zhang, L., Yao, W., Xia, J., Wang, T., & Huang, F. (2019). Glucagon-induced acetylation of energy-sensing factors in control of hepatic metabolism. *International Journal of Molecular Sciences*, 20, 1–20.

Zhang, X., Yang, S., Chen, J., & Su, Z. (2019). Unraveling the regulation of hepatic gluconeogenesis. *Front Endocrinol*, 9, 1–17. https://doi.org/10.3389/fendo.2018.00802

Zhang, Y., Thai, K., Jin, T., Woo, M., & Gilbert, R. E. (2018). SIRT1 activation attenuates α cell hyperplasia, hyperglucagonaemia and hyperglycaemia in STZ-diabetic mice. *Nature Scientific Reports*, 8, 13972. https://doi.org/10.1038/s41598-018-32351-z

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