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Review

Fatty acids, lipotoxicity and insulin secretion

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Non-esterified fatty acids (NEFA) serve as an important energy source for most body tissues, particularly during periods of food deprivation, but recent evidence suggests that these same molecules subserve a much broader function in whole body fuel homeostasis by virtue of their ability to act as potent signalling entities in a variety of cellular processes. One such auxiliary role of NEFA is to heighten the responsiveness of the pancreatic beta cell to a variety of insulin secretagogues. Importantly, this fatty acid-beta cell interaction, though designed by nature for physiological purposes, can, under certain circumstances, take on a pathophysiological dimension. Some new developments surrounding this Jekyll and Hyde character of fatty acids will be reviewed briefly below.

NEFA and normal beta-cell function

(i) The case for glucose-fatty acid cross-talk in the control of insulin secretion

It is generally agreed that in order to stimulate insulin secretion, glucose must first enter the beta cell via a glucose transporter and then be metabolized to a point beyond pyruvate in a process initiated by the high K_m enzyme, glucokinase. This in turn is thought to cause an increase in the ATP:ADP ratio, closure of the cell surface K^+_{ATP} channels, cell depolarization and opening of the voltage-sensitive Ca^2 channels, leading to a rise in intracellular Ca^{2+} $[Ca^{2+}]_i$ and activation of exocytosis [1]. Additional mechanisms contribute, however, to the regulation of insulin secretion in the whole animal setting [2]. One of these, referred to as the K^+_{ATP} channel-independent pathway, augments the response to a raised $[Ca^{2+}]_i$ generated through the more classical pathway. A second, referred to as the K^+_{ATP} channel-independent, Ca^{2+}-independent pathway of glucose signalling, appears to involve a GTP-dependent step that is activated through the combined effects of protein kinase A (PKA) and protein kinase C (PKC).

Although details of these partially overlapping signalling systems remain to be worked out, yet another element must now be brought into the discussion. This has to do with the powerful influence of glucose metabolism on the intracellular disposition of fatty acids and the potential role of this interaction in stimulus-secretion coupling. That fatty acids can considerably enhance glucose-stimulated insulin secretion (GSIS) in intact animals and humans was recognized in early studies from a number of laboratories [3–10] but since many interventions that modulate NEFA concentrations also alter glucose uptake [11], it was often felt that changes in insulin sensitivity could explain most of the fluctuations in plasma insulin concentrations. Recent studies specifically designed to monitor insulin secretion patterns following manipulation of the plasma NEFA concentration have, however, generated renewed interest in the importance of these substrates in governing beta-cell function [12, 13; see below].

Efforts to elucidate how fatty acids influence beta-cell function have led to a series of important findings. Thus; (i) in isolated rodent islets, concentrations

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Abbreviations: NEFA, Non-esterified fatty acids; GSIS, glucose-stimulated insulin secretion; 2-BrP, 2-bromopalmitate; 2-BrS, 2-bromostearate; LC-CoA, long chain acyl-CoA; NA, nicotinic acid; NO, nitric oxide; CPT I, carnitine palmitoyltransferase I; ACC, acetyl-CoA carboxylase; MCD, malonyl-CoA decarboxylase; PKA, protein kinase A; PKC, protein kinase C; HIT, Syrian hamster insulinoma; INS-1 cells, insulin-producing cell line derived from rat islets.

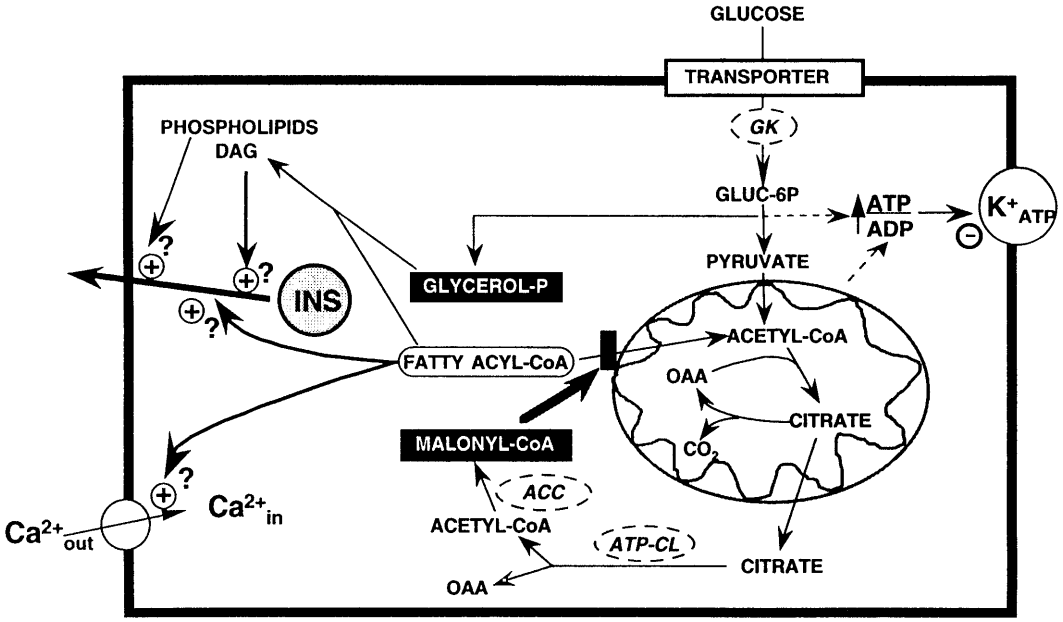

Fig. 1. Potential interactions in the pancreatic beta cell. OAA, oxaloacetate; DAG, diacylglycerol; GK, glucokinase; ATP-CL, ATP-citrate lyase; ACC, acetyl-CoA carboxylase; INS, insulin

of glucose that were stimulatory for insulin secretion were found to suppress the oxidation of long chain fatty acids [14–16]; (ii) agents such as 2-bromostearate (2-BrS) and 2-bromopalmitate (2-BrP), which are known inhibitors of carnitine palmitoyltransferase I (CPT I, the outer mitochondrial membrane enzyme that governs the flow of long chain fatty acids through the mitochondrial beta-oxidation pathway [17, 18]), were shown to stimulate insulin release from perifused islets [15, 19], the Syrian hamster insulinoma (HIT) cell line [20] and the perfused rat pancreas [21]; (iii) it was established that mitochondria isolated from rat islets expressed CPT I activity and, as is the case for all tissues studied to date [18], the islet enzyme could be potently inhibited by malonyl-CoA, the product of the acetyl-CoA carboxylase (ACC) reaction [22]; (iv) of particular interest, exposure of rat islets or HIT cells to glucose and a number of other secretagogues resulted in an increase of the cellular malonyl-CoA content [20, 23, 24]; moreover, in HIT cells this increase was roughly in proportion to the extent of stimulation of insulin release [24]; (v) insulin-producing cell line derived from rat islets (INS-1) cells responded to high glucose concentrations with a major increase in transcriptional activity of the ACC gene [25]; and (vi) exogenous long chain fatty acids appreciably potentiated GSIS from rat islets [15, 26] and HIT cells [20]; in the latter, a concomitant rise in the concentration of long chain acyl-CoA esters was observed.

Taken together, these findings led to the suggestion [27] that stimulus-secretion coupling within the beta cell might use an element of glucose-fatty acid cross-talk analogous to that shown 20 years ago for the control of fatty acid oxidation and ketone body production in liver [28]. The model that has emerged is shown schematically in Figure 1. This can be summarized as an increase in glucose concentration that is sensed by glucokinase, allowing metabolism of the hexose through glycolysis to pyruvate. In the beta cell, just as in liver and other tissues, the C_3 unit can be converted into citrate in mitochondria. Some fraction of the citrate is oxidized in the tricarboxylic acid cycle, generating CO_2 and ATP, while the remainder possibly leaves the mitochondria and is converted in the cytosolic compartment into malonyl-CoA via the sequential action of ATP-citrate lyase and ACC. The increase in malonyl-CoA concentration is expected to suppress CPT I activity, and therefore fatty acid oxidation, resulting in an increase in the cytosolic concentration of long chain acyl-CoA (LC-CoA) which then acts as a signalling molecule for insulin secretion, working in concert with the rise in $[Ca^{2+}]_i$ caused by alterations in the K^+_{ATP} and Ca^{2+} channel activities described earlier. The precise site(s) of action of LC-CoAs in this model remains unclear, although several theoretical possibilities exist. One would be that they react with glycerol-3-phosphate, also derived from glucose metabolism, to form (a) phospholipids that might be required for reworking of the cell membrane during the exocytotic event or (b) a discrete pool of diacylglycerol that serves to activate PKC, an enzyme previously implicated as a player in the insulin secretory process [29]. Alternatively, the LC-CoA might have a more direct effect, such as facilitation of insulin vesicle trafficking [30], alteration of ion channel activity or enhancement of

the docking or fusion of the insulin vesicle or both with the cell membrane (see also below).

It should be noted that the formulation depicted in Figure 1 has not been rigorously validated. Arguments both for and against its operation have been advanced. Two sets of observations would be entirely consistent with the model. The first is that when hydroxycitrate, an inhibitor of ATP-citrate lyase, was added to the medium perfusing the intact rat pancreas, GSIS was greatly attenuated (under the same conditions citrate was without effect [31]). By contrast, addition of palmitate caused a pronounced enhancement of hormone release in response to glucose. When hydroxycitrate and palmitate were combined, however, the rate of GSIS equalled that seen in the presence of the fatty acid alone [31]. In the same report it was shown that hydroxycitrate, but not citrate, blunted the glucose-induced suppression of [^{14}C]-palmitate oxidation. A reasonable interpretation of the findings would be that hydroxycitrate interfered with the generation of malonyl-CoA from glucose, allowing continued oxidation of endogenously generated LC-CoA (regardless of source) and preventing its cytosolic accumulation to a level needed for enhancement of exocytosis. The effect of palmitate could then be explained by its ability to expand the LC-CoA pool directly, thus boosting GSIS despite the continued presence of hydroxycitrate [31]. The second observation supporting the model of Figure 1 was provided by a report in which INS-1 cells were generated that had been stably transfected with a cDNA designed to produce an anti-sense messenger RNA for ACC [32]. This manoeuvre, which successfully lowered the ACC mRNA, protein and activity, also limited the ability of a high glucose concentration to increase the cellular content of malonyl-CoA and to suppress fatty acid oxidation. Concomitantly, insulin release in response to glucose was severely attenuated.

Three lines of evidence which, on their face, are not compatible with involvement of a malonyl-CoA:CPT I:LC-CoA partnership in the regulation of insulin secretion also deserve mention. Firstly the failure to observe any negative effect of HC on GSIS from isolated rat islets [33]. A simple explanation for the discrepancy between this finding and another [31] described above is not readily apparent, although we suspect that it might have to do with differences in the behaviour of batch-incubated islets [33] and islets in situ [31]. Secondly the finding that in patch-clamp experiments with dispersed mouse beta cells, LC-CoA induced a rapid and slowly reversible opening of the K^+_{ATP} channel [34]. Such an effect would be expected to diminish rather than enhance nutrient-induced insulin secretion and was proposed by the authors to be a factor in the genesis of beta-cell glucose insensitivity following prolonged exposure to NEFA (see below). On the other hand, it remains possible that LC-CoA exert opposite acute and long-term effects on stimulus-secretion coupling in the intact beta cell. The third observation comes from studies in which INS-1 cells and isolated rat islets were infected with an adenovirus containing a cDNA encoding the enzyme malonyl-CoA decarboxylase (MCD). In both preparations the transfected cells expressed abundant activity of MCD and this was accompanied by a much reduced capacity of a high glucose concentration to cause an increase in the malonyl-CoA concentration. As a result, glucose-induced suppression of palmitate oxidation was appreciably impaired, but this had no impact on GSIS [35]. Although such findings are difficult to reconcile with the model of Figure 1, it must also be kept in mind that the effects of MCD expression on the various metabolic variables measured were not complete and that small residual perturbations in the malonyl-CoA/CPT I/LC-CoA system induced by glucose might still have been instrumental in the stimulation of insulin release. It is also noteworthy that the fold stimulation of insulin secretion in response to high compared with low glucose concentrations is invariably much lower in cultured beta-cell preparations than in the intact pancreas. As a result, direct comparison of data obtained with the two systems might not always be appropriate.

(ii) Starvation – induced dependence of insulin secretion on NEFA

(a) Studies in rats. For the reasons given above, it seems prudent to delay final judgment on the validity of the malonyl-CoA/CPT I/LC-CoA component of stimulus-secretion coupling until more conclusive experiments have been conducted. This point notwithstanding, an additional line of investigation has now produced unequivocal evidence that under certain circumstances fatty acids play an *indispensable* role in pancreatic beta cell function. These new investigations were prompted by a reconsideration of much earlier work [36] which reported that GSIS from perifused islets of 24 h-fasted rats was greatly reduced when compared with the response of "fed" islets, despite a small difference in the total insulin content of the two preparations. This phenomenon became the subject of many subsequent investigations [37], but a satisfactory explanation proved elusive. In approaching the problem, we reasoned that if the starvation-induced defect in GSIS seen in isolated islets were also to apply in vivo, then when challenged with an intravenous load of glucose a 24 h-fasted rat should display a very poor insulin response relative to that measured in a fed animal, i.e. the fasted rat should in effect be diabetic. To test this unlikely proposition, we carried out a series of experiments on fed and 18 to 24 h-fasted rats that had been previously catheterized and were

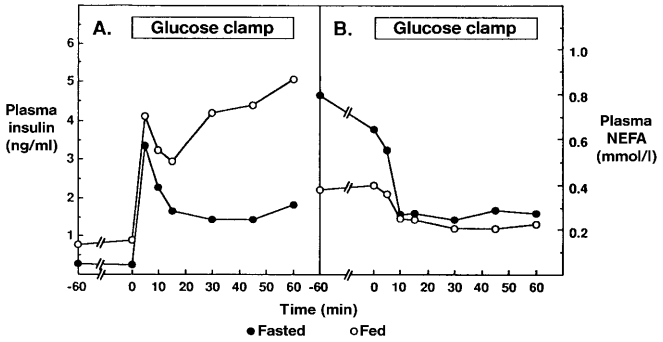

Fig. 2 A, B. Effect of a hyperglycaemic clamp on plasma insulin and NEFA concentrations in fed and 18 to 24 h-fasted rats. At 0 min, the rats received an i. v. bolus of glucose to raise the plasma glucose concentration to approximately 14 mmol/l at + 5 min. Thereafter, the plasma glucose concentration was clamped at approximately 11 mmol/l. Adapted from ref. 38 and reproduced from ref. 56 with permission. To convert ng/ml to pmol/l multiply by 166

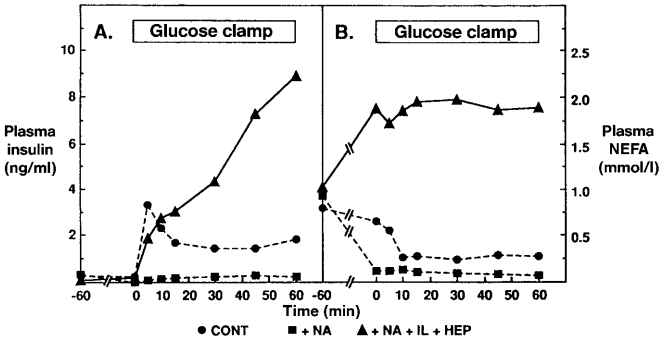

Fig. 3 A, B. Effect of nicotinic acid on glucose-stimulated insulin secretion in 18 to 24 h-fasted rats. Animals were treated as described in Figure 2. In addition, they received vehicle (CONT), nicotinic acid (NA) or NA plus Intralipid (IL) and heparin (HEP) from –60 min onwards. Adapted from ref. 38 and reproduced from ref. 56 with permission. To convert ng/ml to pmol/l multiply by 166

awake and unstressed at the time of study [38]. In each case there was an equilibration period of 60 min at which point the animals received an intravenous bolus of glucose followed by a hyperglycaemic clamp. At the indicated times, blood samples were taken for analysis and quantitatively replaced with a mixture of washed red cells in 10% dialysed fed-rat serum. As seen from Figure 2A, the insulin profile in fed animals showed the typical biphasic form following the glucose challenge. As expected, the basal insulin concentration was lower in the fasted rats. The rapid incremental insulin response to glucose loading was similar in magnitude, however, to that seen in the fed animals (although for reasons that will become clear later, second phase insulin secretion was less robust in the fasted rats) and the antilipolytic effect of insulin was clearly evident in both groups (Fig. 2B). The point to be emphasized is that had this type of experiment been done in an in vitro setting, there would have been a much poorer relative insulin response to glucose in the "fasted" compared with the "fed" pancreas [36, 37 and see below].

The implication from the experiment depicted in Figure 2 was that in the intact fasted rat some factor(s) acted in concert with glucose to allow efficient insulin secretion and, by definition, was absent in previous in vitro studies. Based largely on the studies cited earlier [3–10, 14–16], as well as subsequent contributions by other groups [39–41], we suspected that the critical entity might be the circulating level of NEFA which invariably rises in the fasted state (Fig. 3B). To test this notion, the high plasma NEFA concentration of fasted rats was first lowered by infusion of the antilipolytic agent, nicotinic acid (NA), before giving the glucose load [38]. Remarkably, under these circumstances GSIS was completely abated (Fig. 3A). By contrast, simultaneous infusion of Intralipid (Kabi Pharmacia, Clayton, N. C., USA) plus heparin together with NA, which caused an increase rather than suppression of the plasma NEFA concentrations (Fig. 3B), produced an appreciable first phase and a greatly exaggerated second phase insulin response to glucose (Fig. 3A). Thus, the basis for sluggish second phase insulin release in fasted rats (Fig. 2) became apparent. When the circulating NEFA concentration was not supplemented exogenously, the initial burst of insulin suppressed lipolysis, thus removing the fatty acid stimulus that sustains a high rate of insulin secretion. In this type of study the ketone bodies track with NEFA in terms of their circulating concentration, but the NA-induced loss of GSIS could not be corrected by infusion of acetoacetate plus beta-hydroxybutyrate to replacement levels [38]. Thus, although the ketone bodies are known to be mildly insulinotropic [42–44], the principal component that allowed GSIS to occur in these experiments with fasted rats was clearly the NEFA. Interestingly, when the protocol of Figure 3 was applied to fed animals, GSIS was unchanged regardless of whether the already low concentration of plasma NEFA was further reduced by NA infusion (Fig. 4). Here again maintenance of an artificially high NEFA concentration with Intralipid and heparin caused, however, supranormal rates of insulin secretion. Also to be noted is that in fasted rats results similar to those shown in Figure 3A were obtained when the glucose load was given intragastrically instead of intravenously [38], implying that the permissive effect of circulating fatty acids for GSIS also applies when the fast is terminated naturally.

Of immediate interest was whether all fatty acids are "created equal" in terms of their insulinotropic potency. A clear-cut answer to this question emerged from the experiment depicted in Figure 5 in which 18 to 24 h-fasted rats were again treated with NA to

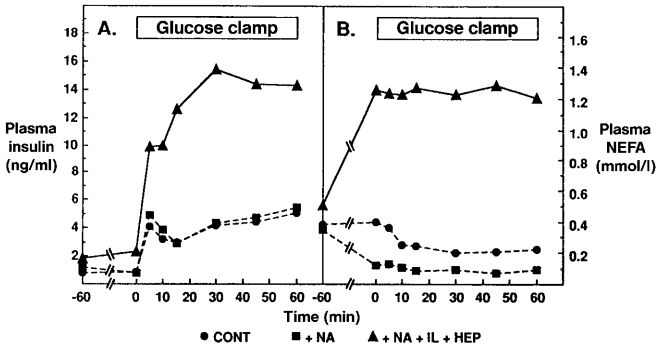

Fig. 4 A, B. Effect of nicotinic acid on glucose-stimulated insulin secretion in fed rats. Details as in Figure 3. Adapted from ref. 38 and reproduced from ref. 56 with permission. To convert ng/ml to pmol/l multiply by 166

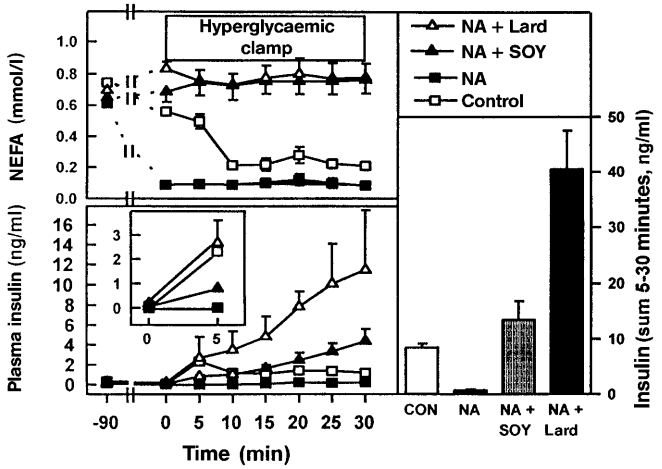

Fig. 5. Effect of nicotinic acid in the absence or presence of heparin plus soybean oil or lard oil infusion on glucose-stimulated insulin secretion in 18 to 24 h-fasted rats. Animals received vehicle (CON), nicotinic acid (NA), NA plus soybean oil (SOY) and heparin or NA plus lard oil (LARD) and heparin from –90 min onwards. Each group received i.v. glucose at 0 min as described in Figure 2. Adapted from ref. 45 and reproduced from ref. 56 with permission. To convert ng/ml to pmol/l multiply by 166

deplete endogenous circulating NEFA. In this case, NEFA were returned to their fasting concentration by coinfusion of heparin together with either soybean oil (the principal constituent of Intralipid used in the studies of Figs. 3 and 4) or the more saturated lard oil that contains a fatty acid mixture closer in make-up to what normally circulated in the test animals [45]. Once again, NA treatment caused almost total abatement of subsequent GSIS. As was true in the studies with Intralipid, the combination of NA and soybean oil partially reinstated the 5 min-insulin response to glucose and caused exaggerated second phase secretion, with the result that total hormone output during the stimulated period completely returned to normal. In sharp contrast, lard oil not only restored a full first phase insulin response to glucose

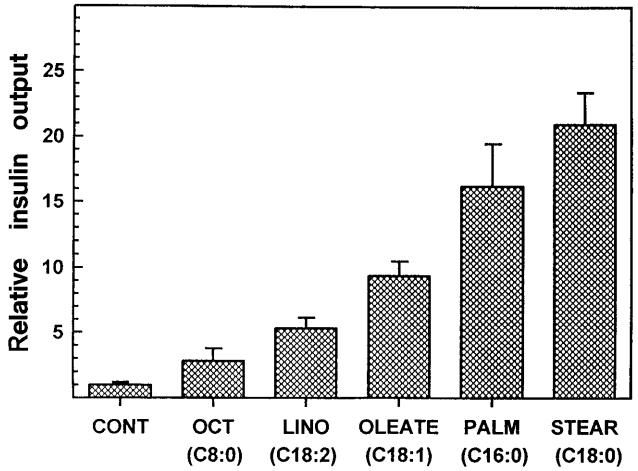

Fig. 6. Effect of different fatty acids on glucose-stimulated insulin secretion from the perfused pancreas of 18 to 24 h-fasted rats. Pancreata were perfused initially with 3 mmol/l glucose and then for 40 min with 12.5 mmol/l glucose in the absence or presence of the indicated fatty acid at a concentration of 0.5 mmol/l (with 1% albumin). The absolute quantity of insulin secreted during the high glucose period was 6.7 ng and is set at unity. OCT, octanoate; LINO, linoleate; PALM, palmitate; STEAR, stearate. Adapted from ref. 45 and reproduced from ref. 56 with permission

but also magnified second phase secretion which now exceeded even that seen with soybean oil infusion. Thus, for the same *total* concentration of NEFA in the circulation, the more saturated species rendered the pancreatic beta cell far more responsive to glucose.

To assess the relative effectiveness of individual fatty acids it was necessary to turn to an in vitro system. Accordingly, GSIS from the perfused pancreas of fasted rats was measured in the presence of different types of fatty acid, all used at a fixed concentration of 0.5 mmol/l in the context of 1% albumin (Fig. 6). Whereas octanoate ($C_{8:0}$) was modestly stimulatory, linoleate ($C_{18:2}$) was much more so. Particularly striking was the finding that as the number of double bonds in the C_{18} fatty acid was reduced, the insulinotropic potency increased dramatically, such that stearate ($C_{18:0}$) caused a 21-fold enhancement of hormone output over that seen in response to 12.5 mmol/l glucose alone. Palmitate ($C_{16:0}$) was almost as effective as stearate (fold stimulation, ~ 16) and, though not shown in the figure, introduction of a single *cis* double bond (to form palmitoleate, $C_{16:1}$) reduced this value to ~ 3 [44]. A *cis*→*trans* switch of the double bond in the $C_{16:1}$ and $C_{18:1}$ species had little impact on their potency [45]. Qualitatively similar results were obtained when the pancreas came from fed rats, and, importantly, in both nutritional states basal (3 mmol/l glucose) insulin secretion was also massively stimulated by the fatty acids, especially the saturated variety [45]. Several fundamental questions are now raised. For example:

1.) How do circulating fatty acids exert this profound influence on the beta cell, and is the mechanism related to the postulated involvement of endogenously produced LC-CoA according to the scheme shown in Figure 1? It is still not clear whether the externally provided fatty acid acts as a carboxylic acid or must first be converted into a CoA ester. If the latter, then the same possibilities as those discussed with reference to the model of Figure 1 would apply to how the acyl-CoA so formed acts to enhance the insulin secretory process. What does seem clear is that in this context the fatty acid brings to the beta cell something other than the simple provision of an energy source. We base this conclusion on the fact that octanoate, which is readily oxidized by a carnitine-independent process, was a relatively poor stimulant of GSIS (Fig. 6). In addition, it should be recalled that a variety of CPT I inhibitors, such as 2-BrP, 2-BrS and etomoxir, were all stimulatory towards GSIS [15, 19–21, 31]. To what extent such agents enhance insulin secretion by virtue of their ability to form non-metabolizable CoA esters (the actual inhibitors of CPT I) or because they cause expansion of the natural LC-CoA pool that is critical for efficient hormone secretion, at least short-term (see above), is not yet known. Also to be considered here is the recent suggestion [46] that an additional contributor to fatty acid-induced beta-cell hypersensitivity to glucose might be an acyl-CoA-mediated increase in metabolism of the hexose secondary to activation of phosphofructokinase.

2.) Where in the secretory pathway does the lipid entity act? Although a firm answer cannot yet be given, it is noteworthy that in the fasted rat the dependence of insulin secretion on plasma-borne NEFA is not a phenomenon restricted to the situation where glucose acts as the secretagogue. Indeed, it has recently been established that the NEFA requirement applies to a variety of other secretagogues as diverse in their mode of action as arginine, leucine, glibenclamide (a sulphonylurea) and a depolarizing concentration of extracellular K^+ [46]. This strongly suggests that the fatty acid-generated signal operates at a distal point in the insulin secretory pathway that is common to the action of all of the agents tested.

3.) Why are saturated fatty acids so much more effective than their unsaturated counterparts? Whether this relates to a difference in metabolism of the two types of fatty acid or to a difference in the physicochemical properties of the effector molecules also remains to be worked out.

4.) Why does starvation render the beta cell absolutely dependent upon circulating NEFA in terms of insulin secretion? Again, a simple explanation is not evident. An attractive possibility would be, however, that in the fed state the beta cell contains adequate amounts of the fatty acid-derived component that synergizes with glucose and other secretagogues to elicit insulin secretion, but that this becomes depleted during fasting and is functionally replaced by the increased plasma NEFA concentration. Whether a fasting-induced reduction in glucose metabolism, e.g. a fall in the activity of glucokinase [47], phosphofractokinase [48], pyruvate dehydrogenase [49] or in the glucose-mediated increase of beta-cell cyclic AMP accumulation [50] is an additional factor also warrants further investigation.

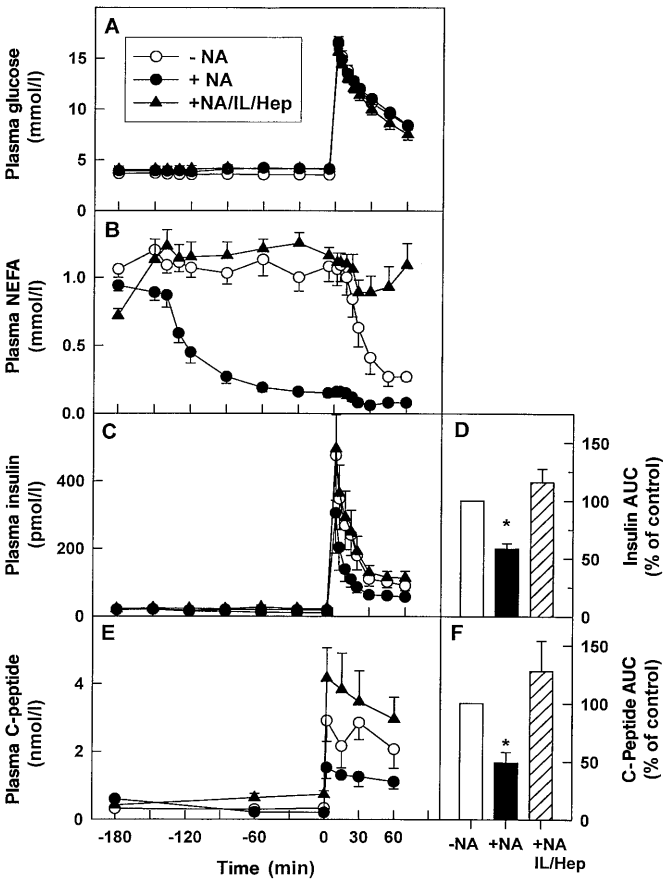

Fig. 7 A, B, C, D, E, F. Effect of nicotinic acid in the absence and presence of lipid infusion on glucose-stimulated insulin secretion in 48h-fasted non-obese humans. When used, Intralipid (IL) and heparin (Hep) were given from –180 min onwards; nicotinic acid (NA) infusion began at –150 min. A glucose bolus was given i. v. at 0 min. AUC, area under curve above baseline. *, $p < 0.05$ compared with -NA control. Reproduced from ref. 12 with permission

(b) Studies in humans. The results obtained in the rat raised the question of what effect food deprivation might have on GSIS in humans. To address this issue, healthy, lean, human volunteers who had fasted for a period of 48 h received a constant infusion of saline from –180 to + 60 min (Fig. 7). At the 0 min time point the plasma glucose concentration was rapidly raised by intravenous bolus to approximately 16 mmol/l and was then allowed to decline. The study was repeated on a second occasion with the inclusion of an infusion of NA from –150 min onwards and on

a third with the further addition of Intralipid plus heparin [12]. In all protocols the basal glucose concentration was clamped at its initial level. As seen from the figure, the effect of prior depletion of circulating NEFA by NA infusion had quite striking effects on beta-cell function. Basal insulin concentrations fell from 19.2 to 10.8 pmol/l (as noted previously in studies with rats and humans [8, 38, 41, 45]) and the incremental areas under the C-peptide and insulin curves in response to the glucose challenge were also reduced by some 50%. All variables returned to normal, however, when the initial plasma NEFA concentration was maintained by coinfusion of the lipid emulsion plus heparin. Clearly, in the 48 h-fasted human, the raised plasma NEFA concentration is essential not only to allow a normal insulin response to an incoming glucose load but also to maintain the low basal concentration of circulating insulin. Similar results were obtained when the fasting period was shortened to 24 h [12]. After a simple overnight fast, lowering of the plasma NEFA concentration with NA had, however, no impact on GSIS, although basal insulin concentrations again fell by about 50% [12].

It thus appears that in healthy, non-obese humans who have fasted for about only 14 h, basal but not stimulated rates of insulin secretion rely upon circulating NEFA. When the fasting period is increased, however, to 24–48 h, about 50% of the beta cell's response to both basal and stimulatory glucose becomes fatty acid dependent. We suspect that this value might have approached 100%, as in the 18 to 24 h-fasted rat (Fig. 3), had the time of starvation been extended to a similar time period for humans, say to 4–5 days.

Interestingly, when overnight-fasted obese volunteers were tested, a different picture was obtained. In this case the NA infusion resulted in a somewhat slower fall in plasma NEFA concentrations, but this was nevertheless associated with a reduction, albeit modest (~ 25–30%), in both basal and glucose-stimulated insulin output [12]. The attenuated responses were of a similar magnitude to the control values in the lean subjects.

(iii) Are fatty acids a factor in the etiology of hyperinsulinaemic states?

The above described studies in obese subjects are entirely in line with previous findings [8] and, in light of newer developments in the field, might provide fresh mechanistic insight into the three hormonal and metabolic disturbances that are hallmarks of obese/Type II (non-insulin-dependent) diabetes mellitus syndromes: (a) insulin resistance, (b) basal hyperinsulinaemia and (c) exaggerated postprandial insulin secretion in the early stages. It is widely held that the hyperinsulinaemia in this setting is a compensatory mechanism for the pre-existing, genetically programmed insulin resistance and represents the body's attempt to stave off the development of impaired glucose tolerance [51, 52]. The connotation here is that insulin resistance begets hyperinsulinaemia. Because it has been difficult to separate these two entities in a temporal sense (they always seem to co-exist), we have suggested that at least two alternative scenarios be considered [53–56]. One would be that subtle increases in insulin secretion give rise to insulin resistance, followed by a vicious cycle in which both abnormalities become pronounced. A second, and probably more likely, formulation is that hyperinsulinaemia and insulin resistance arise simultaneously as a result of some other primary derangement. We and others [55–59] have speculated that this defect(s), which is undoubtedly polygenic and heterogeneous in character (and possibly represents in a functional sense the postulated "thrifty gene" [60]), resides somewhere in the leptin signalling pathway of the central nervous system and results in a decreased ratio of sympathetic: parasympathetic outflow to peripheral tissues. As noted elsewhere [55], such a perturbation might be expected to have widespread effects, including increased tissue malonyl-CoA concentrations, a decreased capacity for fatty acid oxidation, a lowering of energy expenditure (possibly exacerbated by diminished expression of one or more of the growing family of uncoupling proteins [61]), and hyperphagia. This constellation of events would likely promote the accumulation of fat not only in adipose tissue but also in other sites. There is now a strong body of circumstantial evidence that when this occurs in the muscle cell, insulin-mediated glucose uptake is compromised [54, 62–67], perhaps through a negative interaction between LC-CoA (whose intracellular concentration tends to parallel that of triglyceride [66]) or some other esterified fatty acid species [68] and the GLUT-4 translocating machinery. Whatever the precise mechanism, the net effect would be insulin resistance. What has remained enigmatic is how, in circumstances such as this, the beta cell seems to be able to sense the problem in muscle and to respond by secreting an additional complement of insulin to match the degree of resistance. Note that this often occurs both in the basal state and after a glucose challenge, even though the absolute blood glucose concentration is no higher in the insulin resistant than in the normal person who, by comparison, secretes much less insulin [69, 70]. The implication is that in the prediabetic, obese, insulin resistant state the individual beta cells have become hypersensitized to glucose or that islet mass has expanded [71] to a degree commensurate with the extent of muscle insulin resistance. An attractive explanation would be that a common factor has come into play at both sites, namely, accumulation of fat which in muscle brings about insulin resistance and in the beta cell provides the putative lipid signal discussed earlier for enhancement of GSIS [8, 55, 71, 72]. In this context, it is noteworthy that after

a mixed meal, insulin resistant people maintain an inappropriately high plasma NEFA concentration [69]. Also, saturated fatty acids, which for some time have been suspected to contribute to the genesis of insulin resistance more than do their unsaturated counterparts [62], are now recognized as having a greater insulinotropic potency (see above).

If a modest accumulation of fat in muscle and the beta cell is instrumental in decreasing insulin sensitivity and proportionally enhancing GSIS, it follows that lowering of the triglyceride concentration in both sites should have the opposite effects. There is increasing evidence to support this view. Thus, insulin sensitivity in rats and humans has been found to relate directly and inversely to the total muscle triglyceride as measured in biopsy samples [64, 66, 67]. Furthermore, in humans the correlation is much stronger when intramyocellular fat is the measured variable [73]. With regard to the beta cell, induction of hyperleptinaemia in normal rats was found to cause total depletion of islet triglycerides and complete abrogation of insulin secretion from the perfused pancreas in response to glucose and arginine; the loss of beta-cell function was immediately restored by addition of fatty acids to the perfusate [74]. Also, tight positive correlations were found between the triglyceride content of skeletal muscle, liver and whole pancreas and variables such as the plasma insulin concentration, beta-cell function and insulin resistance in a variety of rat models with very different fat content [72]. It is thus tempting to speculate that depletion of fat from muscle and the pancreatic islets underlies the well known improvement in insulin sensitivity and lowering of insulin concentrations in obese, insulin resistant subjects who successfully lose weight by caloric restriction or exercise or both. The exciting possibility exists that pharmacological agents designed to produce these same effects will be helpful in preventing Type II diabetes in people at high risk of developing the disease.

NEFA and abnormal beta-cell function

The ability of circulating NEFA to sustain basal beta-cell function in the fasted state and to assure efficient nutrient-stimulated insulin secretion when the fast is terminated must both be considered physiological phenomena. Even if islet lipids are also instrumental in the hypersecretion of insulin in the obese state, this too would have to be viewed, at least in one sense, as a beneficial adaptation, since it presumably protects against glucose intolerance. Such an adaptive response is shown in experiments [41], in which lipid infusion for 48 h to normal humans induced insulin resistance and chronic hyperglycaemia accompanied by hypersecretion of insulin. Evidence is mounting, however, that there is a darker side to the fatty-acid beta-cell interaction. For example rats were subjected to a large infusion of Intralipid, such that plasma NEFA concentrations were raised to the 1–2 mmol/l range, and at different times GSIS measured in the isolated perfused pancreas [75]; whereas the insulin response was accentuated after 3 and 6 h of lipid infusion, it was considerably blunted after 48 h. Differences in the responses of rats and humans to 48 h of lipid infusion might be explained by plasma glucose concentrations, since the rats did not exhibit hyperglycaemia similar to that seen in humans. In separate studies, initial stimulation followed by suppression of GSIS was noted [76] after perifusion of rat islets with 1 mmol/l palmitate (in the context of 3% albumin), but under these conditions the inhibitory effect of the fatty acid was evident after only 2 h. Subsequent studies involving long-term exposure of rat islets to high concentrations of fatty acids [77, 78] showed a consistent pattern, namely, an enhanced insulin secretion at low glucose concentrations, coupled with suppression of proinsulin biosynthesis, reduced insulin stores and an impaired ability of the beta cell to respond to a high glucose concentration. These are characteristic features of the beta-cell dysfunction in human Type II diabetes, as shown, for example in experiments in which obese Type II diabetic patients displayed enhanced insulin secretion in response to a small glucose load, but reduced insulin release when the glucose challenge was increased [79]. These features are also seen in the male Zucker Diabetic Fatty rat. A striking feature of this animal model is a pronounced increase in plasma NEFA and triglyceride concentrations in the prediabetic phase and a sharp increase in islet triglyceride content immediately before the hyperglycaemia appears, which is usually at about 9–11 weeks of age [71]. Furthermore, diet restriction of the animals from 6 weeks of age reduced the hyperlipacidaemia, the hypertriglyceridaemia and the accumulation of fat in the islet. Under these conditions, there was no hyperglycaemia and a substantial improvement of beta-cell function was clearly evident, i.e. the entire phenotype of Type II diabetes was prevented [71]. An increase in triglyceride content that was associated with inhibition of beta cell function was also observed after long-term exposure of rat islets to high levels of fatty acids in vitro [80].

Observations such as these raise the possibility that in individuals genetically predisposed to develop Type II diabetes, years of exposure of the pancreatic islet to raised concentrations of circulating NEFA or VLDL or both might have a deleterious effect on the beta cell (as well as muscle) and thus contribute to its abnormal function [55, 71]. Possible mechanisms underlying this "lipotoxicity" are beginning to emerge from some recent work. One is that NEFA-induced beta-cell dysfunction is mediated by upregulation of inducible nitric oxide synthase and overproduction of NO [81]. In a related study it was found

that NO production by rat islets exposed to the cytotoxic agent, interleukin-1β, varied directly and cell viability inversely with the islet triglyceride content [82]. Moreover, agents that lowered the islet triglyceride concentration, such as leptin and troglitazone, protected against interleukin-1β-induced NO production and cytotoxicity [82]. The additional possibility has been raised that fat-laden islets undergo an accelerated rate of apoptosis and that increased ceramide synthesis (driven by excess availability of NEFA) is a mediator in this process [83]. Interestingly, apoptosis appears to play a part in the failure of the beta cell to compensate for insulin resistance in the male Zucker Diabetic Fatty rat [84]. Collectively, these studies in the rat strongly implicate an overabundance of islet lipid as an important factor in the demise of the beta cell in human Type II diabetes. Whether this interspecies extrapolation is justifiable remains, however, to be established.

Conclusion

In the foregoing sections we have attempted to develop the theme that fatty acids have "good, bad and ugly" effects on fuel homeostasis in addition to their well established role as an energy source for body tissues. Our focus has been on the pancreatic beta cell where it is becoming clear that these lipid molecules have both physiological and pathophysiological roles depending upon the circumstances. Thus:

1.) It appears that starvation results in loss from the beta cell of a fatty acid-derived entity that is critical for stimulus-secretion coupling.
2.) In the fasted rat, were it not for the raised plasma NEFA concentration, the pancreas would be largely blind to glucose when the fast is terminated.
3.) The situation appears to be similar in humans, but over an expanded time scale of food deprivation.
4.) The same substrates (NEFA), whose plasma concentration rises (appropriately) during starvation, are instrumental in their own disappearance with refeeding (by virtue of their synergism with glucose in stimulating insulin secretion which, in turn, suppresses lipolysis in adipose tissue).
5.) The insulinotropic potency of fatty acids increases with their degree of saturation.
6.) At any given time, insulin secretion will be governed not only by the blood glucose concentration, but also by the prevailing *concentration* and *nature* of the circulating fatty acids.
7.) An excessively high ratio of saturated to unsaturated NEFA in the blood might promote hypersecretion of insulin and thus contribute to the hyperinsulinaemia, dyslipidaemia and insulin resistance that accompany the overconsumption of dietary saturated fat.
8.) In the early stages of obesity/Type II diabetes syndromes, insulin resistance in the muscle cell is matched by hypersecretion of insulin from the beta cell. The possibility is now raised that the linking mechanism stems, at least in part, from accumulation of lipid at both sites.
9.) How and at which point in the insulin secretory process the lipid entity works is still not clear. The site of action appears, however, to be at a late step that is common to a wide variety of secretagogues.
10.) Long-term exposure of the beta cell to excessive quantities of NEFA or VLDL or both might be a factor in the beta-cell dysfunction of Type II diabetes.

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