Are increased plasma non-esterified fatty acid concentrations a risk marker for coronary heart disease and other chronic diseases?

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INTRODUCTION

Among the strongest of the many risk markers for coronary heart disease (CHD) are elevated concentrations of blood lipids, cholesterol and (more controversially) triacylglycerol (TAG). It is not usual to associate increased concentrations of another plasma lipid constituent, the non-esterified fatty acids (NEFAs), with increased risk of cardiovascular disease. In fact, the condition is not even credited with a common name, although hyperNEFAnaemia has been proposed [1] (we prefer the term hyperNEFAemia which rolls off the tongue more easily). However, there is increasing evidence for a central role of increased plasma NEFA concentrations in the ‘insulin resistance syndrome’ or metabolic syndrome [2], and in the associated predisposition to CHD and other chronic diseases including non-insulin-dependent diabetes mellitus (NIDDM), hypertension and possibly even some forms of cancer.

One reason for the lack of appearance of elevated NEFA concentrations as a risk marker for CHD may be simply that they are not often measured in epidemiological studies. Another reason, however, is that plasma NEFA concentrations are highly variable with time within one individual, according to nutritional state, exercise, smoking and stress level, making them less reliable as metabolic markers in large scale epidemiological studies in which control of such factors may be difficult. In addition, the potential role of NEFAs may be overlooked because of their low plasma concentration in relation to other potential risk markers (Table 1). Such a view ignores their extremely rapid turnover (Table 1). One aim of this review is to bring together evidence suggesting that elevated NEFA concentrations may play an important role, in the hope of drawing attention to the possibility of including them in future studies. The review will concentrate on data from human studies.

REGULATION OF THE PLASMA NEFA CONCENTRATION

The concentration of NEFA in plasma at a given moment is the net sum of several events: (i) production, mainly through lipolysis in fat tissue but also by hydrolysis of TAG in circulating lipids; (ii) re-esterification, some NEFAs formed during lipolysis in adipose tissue are re-esterified to TAG in fat cells; (iii) peripheral utilization, NEFAs are taken up by liver and skeletal muscle and used for either synthesis of TAG to be incorporated into lipoprotein (liver) or oxidation (mainly muscle). Except during exercise, the systemic NEFA concentration is determined largely by the rate of NEFA entry into the circulation: there is little direct regulation of NEFA utilization, at least in the resting state.

The plasma NEFA concentration varies considerably with time, with a pronounced circadian rhythm (Fig. 1). The levels are highest in the morning and decrease gradually during the day; suppression is exaggerated after meals, and there is a gradual increase during the night. While some of this pattern is attributable to meal ingestion (as discussed below), other mechanisms contributing to the diurnal rhythm in circulating NEFA concentration are unknown.

Postabsorptive (overnight-fasted) state

The body's fat stores are almost entirely in the form of TAG in adipocytes. The process of fat mobilization consists of hydrolysis of the stored...
Table I. Typical plasma concentrations and turnover of some major substrates

<table>
<thead>
<tr>
<th></th>
<th>Typical plasma concentration (mmol/l)</th>
<th>Typical daily range in healthy subject (mmol/l)</th>
<th>Typical turnover (μmol min⁻¹ kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEFA</td>
<td>0.3</td>
<td>0.1-0.7</td>
<td>6</td>
</tr>
<tr>
<td>Glucose</td>
<td>5</td>
<td>5-8</td>
<td>10</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>5</td>
<td>4.9-5.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Triglycerol</td>
<td>1.5</td>
<td>1.2-2.0</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Fig. 1. Circadian variation in plasma NEFA concentrations in subjects with (●) and without (○) NIDDM. Redrawn from [37] with permission.

TAG to release NEFA into the circulation. The key enzyme is the intracellular TAG-lipase known as hormone-sensitive lipase (HSL; EC 3.1.1.3). HSL releases two of the fatty acids from TAG; the third is removed by a very active monoacylglycerol lipase which, in contrast to HSL, appears not to be regulated. The major form of regulation of HSL, and the only one investigated in any detail, is reversible phosphorylation. The human enzyme is phosphorylated on serine 552 by cyclic AMP-dependent protein kinase (protein kinase A) [3]. This phosphorylation brings about a 50-fold increase in HSL's lipolytic activity in isolated fat cells, although rather less when the purified enzyme is tested against artificial substrates [3].

Because protein kinase A is activated by elevated cyclic AMP concentrations, lipolysis is stimulated by effectors which increase the activity of adenylate cyclase in adipocytes, leading to formation of cyclic AMP from ATP. Adenylate cyclase is stimulated by hormones acting via cell-surface receptors and G, proteins, especially catecholamines acting via β-adrenoceptors. (Glucagon is lipolytic in some systems but probably not in vivo in humans.) The hormonal regulation of lipolysis in humans has been reviewed recently [4].

Dephosphorylation of HSL occurs when the cyclic AMP concentration falls. The main hormonal regulator of this is insulin, which lowers adipocyte cyclic AMP concentrations by activation of the cyclic GMP-inhibited phosphodiesterase, phosphodiesterase III [5]. The suppression of fat mobilization by insulin occurs at very low insulin concentrations. Estimates of the insulin concentration giving half-maximal suppression of fatty acid release in vivo range from around 2 m-units/l [6] to 15-20 m-units/l [7, 8]. Other regulators bring about the same response (lowering of cyclic AMP) by direct inhibition of adenylate cyclase, including adenosine, prostaglandins of the E-series and catecholamines acting via α-2 adrenoceptors [9]. Adenosine, in particular, probably acts as a local regulator of adipose tissue metabolism.

Longer-term regulation of HSL by regulation of gene expression has not been extensively investigated. Regulation of gene expression is not involved in the acute hormonal stimulation of HSL [10]. The amount of mRNA for HSL increases in situations in which fat mobilization is increased, such as during later starvation (3-5 days) [11] and pregnancy [12] in the rat, and in human cancer patients [13]. Decreased HSL expression was observed after weight reduction in obese subjects [14].

Overall, fat mobilization is stimulated under conditions when adrenergic activity predominates over insulin (e.g. in exercise or starvation); it is suppressed when insulin predominates (e.g. after meals, in the postprandial state) (see Fig. 1).

Postprandial (fed) state

HSL activity is thus suppressed after meals. Teleologically, at this time there is no need for the
Fatty acids as a risk factor

body to mobilize its own fat stores, and the drive is more towards fat storage. Nevertheless, there are conditions under which fatty acids continue to enter the plasma. This process of fatty acid release in the postprandial state has only recently been investigated in any detail, and its regulation and physiological significance are not yet clear. In the postprandial state, the enzyme lipoprotein lipase (LPL; EC 3.1.1.34) in adipose tissue is activated by insulin [15] and possibly also by some gastrointestinal peptide hormones [16]. This enzyme is synthesized within adipocytes (and other parenchymal cells), but is exported to the capillary endothelial cells, where it is attached to the luminal side of the capillary wall and acts on circulating TAG in the TAG-rich lipoproteins (chylomicrons and very-low-density lipoprotein, VLDL). LPL releases fatty acids which may be taken up into the tissue for esterification, and thus storage as TAG. It is more active against chylomicron-TAG than against VLDL-TAG [17]. Thus, after a fatty meal, the rate of action of LPL increases both because of hormonal activation and because of a change in the nature of the substrate presented to it.

The fatty acids released by LPL action are not all taken up into adipose tissue for storage. It has long been clear from animal studies that a proportion (perhaps up to 50%) of the fatty acids released by LPL ‘escape’ tissue capture and enter the systemic circulation [18]. The same is true in humans. Labelled lipoprotein TAG-fatty acids enter the plasma NEFA pool rapidly [19]. If labelled fatty acids are added to a meal, they also enter the plasma NEFA pool directly, at a rate which may contribute nearly 50% of the total NEFA appearance rate in the postprandial period [20]. Studies of fatty acid and TAG handling in human subcutaneous adipose tissue in vivo after meals with different fat contents suggest that, at the time of peak LPL action (3–5 h after the meal), 50–75% of the fatty acids released by LPL may enter the systemic circulation directly [21, 22]. Thus, the fatty acid pattern in the plasma NEFA changes after a meal, to reflect the fatty acids present in the meal [23–25].

Unlike the process of mobilization of adipocyte fatty acids by HSL, regulation of the release of LPL-derived fatty acids has not been extensively investigated. It depends on the activity of LPL, which in turn depends on the insulin response to the meal and the sensitivity of LPL activation to insulin (and perhaps other hormones). It probably depends more, however, on the intracellular handling of fatty acids. Suppression of HSL by insulin in the postprandial period, coupled with an insulin-stimulated increase in esterification of fatty acids in adipose tissue [8, 21, 26], leads to a concentration gradient down which fatty acids appear to flow from capillaries to cells [21] (Fig. 2). Fatty acids are transported across the adipocyte cell membrane by a fatty acid transporter protein [27]. It might be thought a priori that the process of transport of fatty acids into cells should be stimulated by insulin, but the opposite appears to be true [28]: the regulatory significance of this step is not yet clear. There might also be regulation by the availability of intracellular fatty acid binding proteins and acyl-CoA binding protein; although there is as yet no clear evidence for this as a short-term regulatory mechanism, these proteins are induced in various tissues in response to a high-fat diet [29].

As well as insulin, other factors may increase fatty acid uptake into cells and subsequent esterification. Among these is the so-called acylation stimulating protein. Acylation stimulating protein is a fragment of an adipocyte product derived by proteolytic cleavage [30], and this activation process appears to be stimulated by the presence of chylomicrons [31]. Thus, a local regulatory system acts to ensure fatty acid removal from the site of LPL action, perhaps in order to minimize product inhibition of LPL (Fig. 2).

Despite the factors operating to ensure uptake of fatty acids derived from LPL action, LPL-derived fatty acids may contribute a substantial proportion of fatty acid release in the postprandial state, as discussed above. We have recently shown that this is particularly so when subjects eat successive fat-containing meals, rather than the single meal after an overnight fast on which much metabolic investigation is based. Ingestion of a second meal 5 h after the first was shown to produce a very rapid and

Fig. 2. Regulation of fatty acid movement in adipose tissue in the postprandial period. Fatty acids (FAs) are generated by the action of LPL on TAG-rich lipoproteins (chylomicrons or VLDL). Cellular uptake of these fatty acids is stimulated by insulin and by acylation stimulating protein (ASP). Concomitant fatty acid release by the action of HSL on intracellular TAG is normally suppressed by insulin. The extracellular fatty acids may have a negative-feedback effect on LPL activity. Those not taken up into cells may remain transiently attached to lipoprotein remnants (a suggested activator of coagulation Factor XII) but eventually become attached to plasma albumin and enter the plasma NEFA pool. See text for further details.
substantial influx of chylomicron-TAG into the circulation. These chylomicrons contained fat from the first meal. This rapid chylomicron-TAG release was accompanied by maintenance of the plasma NEFA concentration, which is normally suppressed after a single meal. Investigation of the profile of individual plasma NEFAs suggested that chylomicron-TAG fatty acids were contributing substantially [32].

Since, in Western societies, a large part of the day is spent in the postprandial state, and the consumption of successive fatty meals separated by a few hours is a common pattern, the contribution of LPL-derived fatty acids to the diurnal plasma NEFA profile may be considerable.

PATHOLOGICAL REGULATION OF THE PLASMA NEFA CONCENTRATION

Typical plasma NEFA concentrations in different physiological and pathological states are shown in Table 2.

Stress states

Adipose tissue TAG hydrolysis by HSL is activated by catecholamines. Thus, fat mobilization and plasma NEFA concentrations increase in stress states, such as mental stress [38, 42], exercise [43], surgery [44] and physical trauma [40]. It has been argued [45, 46] that fat mobilization in these states reflects, in evolutionary terms, the need to supply fuel for 'fight or flight', but that the consequent rise in plasma NEFA concentration (when the fatty acids are not utilized in muscular work) may be maladaptive. Thus, extremely high plasma NEFA concentrations and an associated rise in plasma TAG concentration have been observed in racing drivers [39].

Obesity, NIDDM and insulin resistance

It has long been recognized that plasma NEFA concentrations are elevated in obese subjects compared with lean subjects [36]. This has been ascribed to the increased adipose tissue mass [47]. Measurements of the rate of NEFA turnover suggest that the rate of NEFA release per unit weight of body fat is reduced in the obese, but that the whole-body rate of NEFA production is still increased [48, 49]. Of crucial importance is probably the fact that lean body mass, responsible for the

<table>
<thead>
<tr>
<th>State</th>
<th>n</th>
<th>Plasma NEFA (mean or median ± SD) (μmol/l)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal postabsorptive (10 h fast)</td>
<td>310</td>
<td>290 ± 180</td>
<td>[33]</td>
</tr>
<tr>
<td>12 h fast*</td>
<td>19</td>
<td>650 ± 170</td>
<td>[34]</td>
</tr>
<tr>
<td>36 h fast</td>
<td>19</td>
<td>1110 ± 300</td>
<td>[34]</td>
</tr>
<tr>
<td>72 h fast</td>
<td>19</td>
<td>1260 ± 260</td>
<td>[34]</td>
</tr>
<tr>
<td>2 h after 75 g of glucose</td>
<td>310</td>
<td>50 ± 45</td>
<td>[33]</td>
</tr>
<tr>
<td>During insulin infusion,</td>
<td>8</td>
<td>20 ± 20</td>
<td>[26]</td>
</tr>
<tr>
<td>35 m-units min⁻¹ m⁻¹ (90 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise: 2 h</td>
<td>5</td>
<td></td>
<td>[35]</td>
</tr>
<tr>
<td>25% VO₂ max</td>
<td></td>
<td>1170 (SD not stated)</td>
<td></td>
</tr>
<tr>
<td>65% VO₂ max</td>
<td></td>
<td>1120 ± 270</td>
<td></td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal weight</td>
<td>8</td>
<td>480 ± 150</td>
<td>[36]</td>
</tr>
<tr>
<td>'Moderate'</td>
<td>8</td>
<td>611 ± 140</td>
<td></td>
</tr>
<tr>
<td>'Gross'</td>
<td>8</td>
<td>810 ± 210</td>
<td></td>
</tr>
<tr>
<td>NIDDM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>330 ± 90*</td>
<td>[37]</td>
</tr>
<tr>
<td>'Mild'</td>
<td>9</td>
<td>370 ± 170</td>
<td></td>
</tr>
<tr>
<td>'Severe'</td>
<td>9</td>
<td>740 ± 140</td>
<td></td>
</tr>
<tr>
<td>Public speaking</td>
<td>21</td>
<td>960 ± 290</td>
<td>[38]</td>
</tr>
<tr>
<td>Race driving</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>16</td>
<td>1720 ± 100</td>
<td>[39]</td>
</tr>
<tr>
<td>After</td>
<td>16</td>
<td>1370 ± 280</td>
<td></td>
</tr>
<tr>
<td>Acute trauma (moderate severity)</td>
<td>76</td>
<td>910 ± 600</td>
<td>[40]</td>
</tr>
<tr>
<td>Acute myocardial infarction</td>
<td>11</td>
<td>1090 ± 530</td>
<td>[41]</td>
</tr>
</tbody>
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NEFA liberation: thus, the rate of NEFA turnover per unit of lean body mass is increased [49, 50]. A specific impairment of NEFA oxidation in skeletal muscle in obesity (particularly of the abdominal variety) [51] would augment the elevation in NEFA concentration.

The elevation of NEFA concentrations in obesity may also relate to the insulin resistance characteristic of that condition. A number of studies show that the ability of insulin to suppress NEFA release in vivo is diminished in obese subjects [20, 49, 50, 52]. This appears to reflect alterations in the insulin sensitivity of both lipolysis and fatty acid re-esterification [49, 52].

An elevated plasma NEFA concentration is also a characteristic of non-obese subjects with insulin resistance [33, 53]. It should be pointed out in this context that it is extremely difficult to disentangle cause and effect in the relationships between circulating NEFA concentrations and insulin sensitivity, since insulin resistance may lead to elevated circulating NEFA concentrations as discussed above, whereas elevated NEFA concentrations may lead to diminished sensitivity to insulin (discussed below) [54, 55]. Thus, in principle there may be a self-perpetuating cycle between insulin resistance and elevated plasma NEFA concentrations. It is interesting that novel oral antidiabetic therapies which appear to act by improvement of sensitivity to insulin also have a marked plasma NEFA-lowering effect [56, 57].

When insulin action is deficient, as in NIDDM, it is not surprising to find elevated plasma NEFA concentrations [37, 58] (Fig. 1). These persist in both postabsorptive and postprandial states [37]. Suppression of fat mobilization by infused insulin may also be impaired [59]. Again, a defect in skeletal muscle NEFA oxidation [60] may accentuate defects in the regulation of lipolysis.

The conditions of obesity, insulin resistance and NIDDM are all strongly associated with an increased risk of CHD. We shall discuss further the links between elevated NEFA concentrations and risk of CHD, but it is important to mention here our own belief that an important aspect of this link is the dys-regulation of NEFA concentrations in the postprandial period, when the body's fuel economy is normally geared to fat storage and oxidation of carbohydrate [54].

Effects of elevated NEFA concentrations

On glucose metabolism and sensitivity to insulin. It has been recognized since the work of Randle and colleagues in the 1960s [61] that there is normally an inverse relationship between the use of fat and carbohydrate as metabolic fuels, and that inappropriate elevation of the supply of fatty acids may lead to impairment of glucose utilization. This has been amply documented in the intervening years, largely by studies in which the plasma NEFA concentration is either elevated by infusion of a lipid emulsion (e.g. [62]) or reduced pharmacologically [63].

A number of mechanisms, both acute and chronic, link NEFA supply and impairment of glucose utilization. Randle et al. [61] showed that fatty acids were oxidized preferentially over glucose by cardiac and skeletal muscle. The mechanism was shown to be increased production of acetyl-CoA, which through a series of feedback inhibitory steps reduced glucose uptake and oxidation. In addition, the supply of NEFA to the liver is an important determinant of the rate of hepatic glucose production [62, 64]. Thus, elevated NEFA concentrations, particularly in the postprandial period when they are usually suppressed, lead to inappropriate maintenance of glucose production and impairment of glucose utilization, i.e. to impaired glucose tolerance. These mechanisms may be an important causal part of the chain of events leading from obesity to NIDDM [65, 66]. The progression to NIDDM may be accentuated by suppressive effects of high NEFA concentrations on insulin secretion [67], or even 'toxicity' of NEFA to pancreatic β-cells [66]. An association between elevated plasma NEFA concentrations and risk of development of NIDDM has been noted in Pima Indians, and attributed to a combination of these mechanisms [68].

A further mechanism links increased NEFA supply and insulin resistance. Increased delivery of NEFA to the liver may reduce insulin binding to hepatocytes [69] and therefore inhibit hepatic insulin clearance. Since the liver normally removes around 40% of insulin secreted from the pancreas, this may have a large impact on peripheral insulin concentrations. Thus, elevated NEFA concentrations lead to acute hyperinsulinaemia, and in the longer term (through down-regulation of receptors) to insulin resistance.

On lipid metabolism. Elevated NEFA concentrations have a number of potential effects on lipid metabolism. Probably the most important is that the rate of NEFA delivery to the liver is a major determinant of hepatic VLDL-TAG secretion [70, 71]. Plasma NEFAs are the major substrate for hepatic TAG synthesis, and there is a close correlation between NEFA and VLDL-TAG concentrations or turnover rates [70, 72]. Although this might be seen as a 'mass-action' effect of increased substrate supply, the mechanism is probably more complex. It seems that newly synthesized hepatic apolipoprotein-B (apoB) is normally partitioned between intracellular degradation and secretion as VLDL. The availability of lipid, which enables the nascent apoB polypeptide chain to adopt a certain conformation, directs the protein away from the degradative pathway [73, 74]. Thus, increased NEFA availability not only increases VLDL-TAG secretion, but also the number of VLDL particles.
secreted (each of which contains one molecule of apoB).

Again, effects in the postprandial period may be particularly important. Insulin has an acute suppressive effect on hepatic VLDL secretion [73, 75, 76]. This makes sense teleologically. In the postprandial period, chylomicrons enter the circulation and compete with endogenous VLDL for clearance by LPL in peripheral tissues [17, 77]. Insulin, secreted in response to a meal, will suppress both NEFA supply to the liver and VLDL output, thus reducing the competition for clearance and minimizing the postprandial rise in TAG concentration. Failure to suppress the NEFA supply normally in this period may lead to sustained VLDL production and impaired clearance of TAG-rich lipoproteins in the postprandial period. The magnitude of postprandial lipoaemia is closely associated with coronary artery disease [78–80].

As mentioned earlier, plasma NEFAs arise in the postprandial period both from intracellular lipolysis and from the action of LPL in capillaries. It has been argued that a failure of entrapment of fatty acids in adipose tissue during the action of LPL on chylomicon-TAG, which may itself be caused by a dysfunction of the acylation stimulating protein system, could be an important mechanism leading to increased VLDL secretion [74]. A further consequence would be increased production of LDL particles (since VLDL is the precursor of LDL), but not necessarily an elevated LDL-cholesterol concentration, since in the presence of high VLDL-TAG concentrations LDL particles become lipid-depleted and thus more dense. The resultant syndrome is characterized by an elevated total apoB concentration in the plasma or ‘hyper-apoB’, which is associated with an increased risk of CHD [81]. The observation of elevated postprandial NEFA concentrations in patients with familial combined hyperlipidaemia [82] also fits with such a mechanism. It is particularly interesting that such patients have reduced HSL activity in adipose tissue [83], perhaps representing compensation for their decreased ability to take up fatty acids into adipose tissue in the postprandial state. Alternatively, an HSL defect might be primary in this condition, and inhibition of the uptake of fatty acids into adipose tissue a compensatory event.

On myocardial function. It has long been appreciated that high concentrations of NEFA may have acute adverse effects on myocardial function [84]. Long-chain fatty acids appear to cause an increase in intracellular Ca2+ which may lead to arrhythmias, conduction disturbances and myocardial damage [85]. It has been proposed that the increase in intracellular Ca2+ is mediated by the accumulation of long-chain acylcarnitine [84]. It should be noted, however, that such effects may be fatty acid specific and there is evidence that n-3 polyunsaturated fatty acids may exert a myocardial stabilizing effect [86]. Since plasma NEFA concentrations may be highly elevated in patients with acute myocardial infarction [41, 84], it has been suggested that they provide one mechanism precipitating the onset of ventricular fibrillation [84]. High NEFA concentrations are also observed in patients in accidental hypothermia [87], and since myocardial stability is already compromised at low temperatures, they may again tip the balance towards the onset of dysrhythmia. An additional mechanism for NEFA inhibition of myocardial function could be inhibition of adenylyl cyclase, as demonstrated in human adipocytes [88], since adenylyl cyclase is an important regulator of cardiac contraction.

On haemostasis. Elevated NEFA concentrations have been associated with increased thrombosis [89]. An increased thrombotic potential is strongly associated with risk of CHD. In particular, elevated plasma concentrations of fibrinogen and of Factor VIIc (coagulant activity of Factor VII) are consistently found to be associated with CHD [90, 91]. Factor VIIc is increased by fat ingestion, both acute and chronic (i.e. by a high-fat diet) [91, 92]. In experiments in which fat loads have been administered, the activation of Factor VII occurs in the postprandial period, and is related to elevated plasma NEFA rather than TAG concentrations [93, 94]. It has been suggested that fatty acids liberated during LPL action are transiently attached to the surfaces of lipoprotein particles undergoing lipolysis (e.g. chylomicrons), and that these fatty acids present a negatively charged surface which is responsible for Factor XII activation, leading subsequently to activation of Factor VII [95, 96]. It has been suggested that fatty acids liberated during the action of LPL are transiently attached to the surfaces of lipoprotein particles undergoing lipolysis (e.g. chylomicrons), and that these fatty acids present a negatively charged surface which is responsible for Factor XII activation, leading subsequently to activation of Factor VII [95, 96]. Saturated fatty acids, which form a more rigid surface, appear to be the most likely to cause this effect in vitro [95]. However, there are some apparent discrepancies in the literature on this point in vivo. In acute meal studies, activation of Factor VII has been linked to individual plasma NEFAs, either stearic acid [94] or the polyunsaturated linoleic acid [93]. In dietary experiments a diet rich in stearic acid leads to lower levels of Factor VIIc than diets enriched in other saturated fatty acids [97].

Fibrinolysis may also be affected. There is also an association between plasma NEFA concentrations and plasminogen activator inhibitor 1, which is thought to contribute to the elevation of plasminogen activator inhibitor 1 concentration, a known risk marker for CHD, in obesity [98]. There is also a direct inhibitory effect of long-chain fatty acids on plasmin activity [99].

On steroid sex hormones. NEFAs are carried in the plasma bound to albumin, a common transport protein for a number of hydrophobic substances. When the plasma NEFA concentration increases, other hydrophobic substances can be displaced by fatty acids from their albumin binding sites; this has been shown for tryptophan [100], warfarin and other hydrophobic drugs [101]. The steroid sex hormones are also carried partly bound to albumin, and partly to the sex-hormone-
binding globulin. Concentrations of sex-hormone-binding globulin are reduced in obesity and other insulin-resistant conditions [102, 103], and an increased proportion of the steroid sex hormones will be carried bound to albumin. In addition, oestrogen concentrations tend to be elevated in concentrations in obesity, brought about by such insulin-resistant conditions [102, 103], and an increased proportion of the steroid sex hormones in adipose tissue [106, 107].

The elevated NEFA concentrations in obesity or insulin resistance may then be responsible for displacement of sex hormones from albumin, and an increase in the proportion free in the plasma. It is the free component which is biologically active. It has been suggested that elevated free oestrogen concentrations in obesity, brought about by such mechanisms, may be a cause of the increased incidence of breast cancer in obese postmenopausal women [108–110]. There is a link between insulin resistance, independent of obesity, and breast cancer [110] which may also be related to such a mechanism, and evidence for increased visceral fat accumulation in breast cancer cases versus controls [111]. Endometrial cancer is another oestrogen-responsive cancer whose incidence is increased in obesity [112], and such mechanisms might also underlie this association. It should be mentioned, however, that some authors do not report such an effect of NEFA on steroid hormone bioavailability [113].

**HETEROGENEITY OF LIPOLYSIS AND CIRCULATING NEFA LEVELS**

Intra-abdominal adipose tissue has some metabolic characteristics that are unique in comparison with other fat depots, as reviewed recently [114]. A significant part of intra-abdominal fat (i.e. the omental and mesenteric regions) is drained by the portal circulation and therefore has direct access to the liver. In addition the lipolytic activity is much higher in fat cells from the omental/mesenteric area than from subcutaneous sites. The mechanism for this site variation resides at the level of hormonal and parahormonal regulation of adipocyte lipolysis (Fig. 3). The antilipolytic action of insulin, adenosine and prostaglandins is more pronounced in the subcutaneous fat cells, whereas the lipolytic action of catecholamines is greater in the visceral adipocytes. These differences seem to be due to site variations in agonist binding and signal transduction of the corresponding receptors; for example, insulin receptor binding, autophosphorylation and signalling through the phosphatidylinositol 3-kinase pathway are decreased in omental compared with subcutaneous fat cells [114]. As a consequence of these regional variations in lipolysis the liver will be more exposed, in relative terms, to NEFA than will the rest of the body. Elevated portal NEFA will influence hepatic lipoprotein production, gluconeogenesis and insulin clearance according to the mechanisms discussed above. Indeed, portal NEFAs have been considered as a risk factor for cardiovascular disease and diabetes [115].

It is now well established that abdominal obesity is closely associated with CHD. The ‘portal’ fat mass constitutes about 20% of total fat mass in abdominally obese subjects [116]. Taking into account the enlargement plus enhanced lipolytic activity of ‘portal fat’ it has been estimated that it may contribute at least 50% of total circulating NEFAs in abdominal obesity [117]. The contribution may be even greater when upper-body obesity is accompanied by the insulin resistance syndrome. In this situation, catecholamine-induced lipolysis is decreased in subcutaneous adipose tissue [118] but increased in ‘portal’ adipose tissue [119], which further enhances the NEFA gradient in portal versus systemic venous circulation. The major mechanisms responsible for these regional variations in adipocyte function seem localized at the level of β-adrenoceptors and HSL. The functions of the β2-adrenergic receptor and HSL are decreased in subcutaneous adipocytes whereas the function of the recently discovered β1-adrenergic receptor is increased in the ‘portal’ fat cells of subjects with signs of the insulin resistance syndrome [118, 119].

It must be borne in mind, however, that these ideas on portal NEFA supply largely rely on observations made in vitro. For clinical reasons it is not easy to investigate the portal vein directly. However, investigations in vivo have given indirect evidence for regional differences in lipolytic activity in obese subjects [48].

**EFFECTS OF PARTICULAR FATTY ACIDS**

The discussion has so far concentrated on plasma NEFA without much reference to particular fatty acids. Although individual fatty acids in the diet clearly have major effects on risk of CHD, mediated through their effects on LDL-cholesterol concentrations, it has not been clearly established that
individual plasma NEFAs have different properties in this or any other respect.

When the profile of plasma NEFAs changes after a fat load, it does not uniformly change towards that of the fat administered: there is a tendency for saturated fatty acids to be over-represented [25, 120]. It has been suggested that there are preferences in the tissue uptake of particular fatty acids at the site of LPL action [25, 120]. The physiological significance of this is not known. There may also be differential mobilization of fatty acids from adipose tissue. This has been clearly shown in rat adipocytes [121] (the more unsaturated are mobilized more relative to the amount stored). During physical exercise in humans the pattern of NEFAs in the plasma changes to become more unsaturated [122], and it has been suggested that this represents a beneficial effect of exercise.

Saturated fatty acids may predispose towards insulin resistance. In humans a relationship has been shown between the nature of the fatty acids in skeletal muscle membrane phospholipids and whole-body insulin sensitivity (the lower the proportion of polyunsaturated fat, the less the insulin sensitivity) [123]. In rats, high-fat diets generally impair insulin sensitivity, but this is abolished if the fat is of the n-3 polyunsaturated variety [124]. However, it is not known whether any of these effects relate specifically to the NEFA fraction. If rat adipocytes are cultured in vitro for 4h in the presence of (non-esterified) saturated fatty acids, a reduction in sensitivity to insulin of glucose transport is observed [125]. However, this is an area in which much more information needs to be gathered in humans.

**FATTY ACIDS AND GENE REGULATION**

Fatty acids have many functions apart from their use as an oxidative fuel. One which has come to light in recent years is their role as a regulator of gene expression. Long-chain fatty acids have been shown to regulate the transcription of a number of genes coding for proteins relevant to adipocyte differentiation and other aspects of lipid metabolism [126, 127]. This is achieved by binding to a regulatory element which has been termed the fatty-acid activated receptor (FAAR), and is homologous to the peroxisome proliferator-activated receptor (PPAR) [126, 128, 129]. However, we feel it is premature to speculate whether any of the adverse effects of fatty acids discussed in this review might be due to fatty acid regulation of gene expression.

**SUMMARY AND CONCLUSIONS**

There are many associations between fat metabolism and risk of CHD, but there seems to be good evidence to suggest that the plasma NEFA concentration may play a central role in some of these associations (Fig. 4). We suggest that failure to regulate the plasma NEFA concentration normally, particularly in the postprandial period and possibly also during catecholamine excess, might be associated with the development of a number of risk factors for CHD including insulin resistance, hypertriglyceridaemia, hyper-apoB and increased coagulant activity. Chronically elevated NEFA concentrations may also underlie (in part) an increased risk of some hormone-sensitive cancers. Acute elevations of NEFA in stress states such as myocardial infarction may have directly deleterious effects on myocardial performance. Increased portal delivery of NEFA may play a crucial role in disturbed hepatic metabolism in the insulin resistance syndrome, and contribute to dyslipidaemia, hyperinsulinaemia and glucose intolerance.

The difficulty in proving the central role of the NEFA concentration is that plasma NEFA concentrations are inherently variable from subject to subject, and also within subjects from day to day. At least in the case of risk of CHD, the plasma apoB concentration may act as an integrated marker of NEFA supply to the liver, and is perhaps the lipid equivalent of haemoglobin A1c in glucose metabolism.

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Fatty acids as a risk factor


