Insulin resistance and the metabolic syndrome—or the pitfalls of epidemiology

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Abstract The clustering of dyslipidaemia, hypertension and glucose intolerance, predominantly in overweight individuals, has been ascribed many names, including syndrome X and the metabolic syndrome. In Reaven's original description of syndrome X, a central aetiological role was attributed to insulin resistance, and this assumption has remained as the dominant paradigm for the metabolic syndrome. There are a number of conceptual problems in such a model, particularly those arising from observations that several novel markers, including measures of endothelial dysfunction and of low-grade inflammation, are as closely related to insulin resistance as are the classic components of the syndrome. Because it is difficult to envisage how these traits might develop as a consequence of insulin resistance, such observations indicate the need for a new paradigm to explain the mechanisms of association better. It has been proposed that a state of low-grade inflammation, consequent upon the production of adipocytokines, particularly from truncal fat, explains the observed relationships between insulin resistance and endothelial dysfunction better than does a model revolving around insulin resistance. Furthermore, the inflammatory cytokines generated from adipose tissue may influence vessel endothelial function without elevations in circulating concentrations. This review alludes to several problems inherent in the epidemiological method in understanding disease

mechanisms. These include crude biological measures, the use of venous systemic fasting samples, imprecision of assays, naive physiological models, simplistic statistical approaches and, without clinical trials, an inability to test causation. Integrated systems biology needs more complex approaches to investigate disease mechanisms, involving cell, organ, whole organism and population studies.

Keywords Adipocytokines · Endothelium · Inflammation · Insulin resistance · Metabolic syndrome

Abbreviations

CRP	C-reactive protein
HOMA	homeostasis model assessment
NO	nitric oxide
PAI-1	plasminogen activator inhibitor-1
UKPDS	United Kingdom Prospective Diabetes Study
VLDL	very-low-density lipoprotein

Introduction

In his 1988 Banting lecture, Reaven described the clustering of cardiovascular risk factors that he termed syndrome X [1]. These comprised resistance to insulin-stimulated glucose uptake, hyperglycaemia, hyperinsulinaemia, increased plasma concentration of very-low-density lipoprotein (VLDL)-triacylglycerol, a low concentration of HDL-cholesterol and high blood pressure. Many other terms have been used to describe such a cluster [2–4], the most common being the metabolic syndrome. This syndrome has been defined in different ways by different bodies [5–7], but all include (central) obesity, dyslipidaemia, hypertension and glucose intolerance. Although these definitions do not attempt to ascribe any aetiological factor to the cluster, and only one

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definition, that of the WHO, includes insulin resistance as one of the components [6], there still remains a widely held belief that insulin resistance plays an important role. As examples, the National Cholesterol Education Program–Adult Treatment Panel III states that the ‘syndrome is closely linked to a generalized metabolic disorder called insulin resistance’ [5], and the International Diabetes Federation Consensus Statement [7] notes that ‘insulin resistance is widely believed to be a central feature of the metabolic syndrome, even though the mechanistic link between insulin resistance and most of the components of the metabolic syndrome is not fully understood’. Thus, the core concept of syndrome X, and by extrapolation the metabolic syndrome, has been the centrality of insulin resistance and/or hyperinsulinaemia, in part determined by obesity, but leading, through various mechanisms, to the clustering of lipid abnormalities, elevated blood pressure and glucose intolerance [1].

There has recently been substantial debate about the value of defining a metabolic syndrome, and even about its very existence, but one focus of the debate has been the issue of its pathophysiology [8–10]. The joint statement by the American Diabetes Association and the European Association for the Study of Diabetes noted that most investigators use the phrase ‘insulin resistance’ to describe the hallmark of the syndrome, but that there was substantial inconsistency in such associations, in part because of measurement problems [8]. Gale commented that ‘insulin resistance, important though it may be, provides an insecure foundation for the metabolic syndrome, to the extent that it is no longer considered a useful (or at least measurable) criterion’ [10]. He continued, ‘A number of associated clinical features congregate in individuals who are at increased risk of heart disease. They cluster together for a reason, and it is important to seek an explanation for this at a pathophysiological level.’

This article will use these critiques as a starting point to look for a reason for the clustering, and in so doing, challenge the evidence for insulin resistance being the central aetiological factor. It will outline the expansion of the syndrome to include several new variables for which, unlike the original components, explanations of pathogenesis based on insulin resistance appear tenuous. It will suggest that one of the major problems in many of the studies of the metabolic syndrome is the problem of confounding, as a consequence of taking inadequate account of body fat mass. It will go on to propose a more feasible model for the observed associations, one in which adipose tissue-derived molecules generate a state of low-grade inflammation, with consequent changes in metabolic and haemodynamic physiology.

This critique of the dominant paradigm for the metabolic syndrome will also have a subsidiary theme. Most of the literature on the metabolic syndrome is based on findings from epidemiological studies, which have, by their very nature,

many limitations in pursuing ideas of causation. The article will outline some of these epidemiological pitfalls, including that of confounding, and will suggest that such approaches may have been responsible in part for the naive physiological models that have been proposed. It will suggest that additional methods are necessary to understand aetiology, and, in turn, to help define treatment strategies. To begin this deconstruction, it is necessary to consider what makes a syndrome.

Clustering of continuous variables

The term ‘syndrome’ is defined as the concurrence of symptoms and signs in a disease, the word deriving from the Greek σύνδρομο and meaning ‘running together’ [11]. In Cushing’s syndrome, where such signs include striae, moon face and buffalo hump, or in AIDS, where the presence of immune deficiency is defined on the basis of particular opportunistic infections, the component parts are categorical variables that are either present or absent. With the metabolic syndrome, however, the variables used in its definition are continuous, quantitative ones. Even though these can be turned into categorical variables, such as hypertension or impaired fasting glucose, by ascribing arbitrary cut-off points, the definition of clustering of the quantitative traits must depend on their closeness of the association, again a continuous quantitative attribute for which shades of proximity can exist. These issues, of how close an association needs to be, and also of how close is the true association between variables, are problematic, as will now be outlined.

Problems in defining clustering of continuous variables

- Strength of even weak associations may achieve significance in large studies
- Poor reproducibility of single measures (biological or assay) will affect correlations
- Hierarchy of relationships may alter when reproducibility is taken into account
- Interrogative statistical methods may show that more than one cluster exists
- Inadequate account of confounding variables may leave significant associations

In his original description, Reaven implicated insulin resistance in syndrome X by showing statistically significant correlation coefficients between the dependent variables and the concentrations of insulin [12]. Table 1 shows similar correlation coefficients between these variables in 1034 healthy men and women aged 40–75 years investi-

Table 1 Correlations of metabolic syndrome variables in the Gooding study ([13, 14], $n=1034$)

	Fasting insulin concentration	2 h insulin concentration	Systolic blood pressure	Diastolic blood pressure	Triacylglycerol	HDL-cholesterol
Fasting insulin concentration	–	0.51***	0.21***	0.17***	0.30***	–0.22**
2 h insulin concentration		–	0.21***	0.18***	0.30***	–0.18***
Systolic blood pressure			–	0.73***	0.24***	–0.04 ^a
Diastolic blood pressure				–	0.20***	–0.07*
Triacylglycerol					–	–0.48***

* $p<0.05$ ** $p<0.01$ *** $p<0.001$ ^a Non-significant correlation

gated in the Gooding study [13, 14]. It is clear that there are significant correlations between all variables and both fasting and 2 h insulin concentrations. But when a study comprises more than 1,000 participants, significant correlations exist even with correlation coefficients as low as 0.07, when less than 0.5% of the variance of the dependent measure is ‘explained’ by that of insulin concentrations.

One issue that is relevant to the strength of a relationship between two variables is that of reproducibility. In most epidemiological studies, variables are measured on a single occasion, although for some measures, such as blood pressure and anthropometry, a mean of two or three observations may be used. But when a measure is made on repeated occasions, there is substantial variability in the observed results, reproducibility decreasing with increasing interval. While part of the explanation for this lies in biological variability, there are also contributions from the method itself, with laboratories recognising variability within- and between-assays, and measures such as waist circumference, blood pressure or skinfold thickness similarly showing intra- and inter-observer variability. We have studied a series of measures in 107 healthy adults on two occasions separated by 3.5 years (J. S. Yudkin, unpublished data; [15, 16]) and correlated values at follow-up with those at baseline. These correlation coefficients vary from $r=0.90$ for BMI to $r=0.46$ for fasting triacylglycerol and $r=0.32$ for fasting insulin concentrations. This repeat measurement variability will influence the correlation between any two measures in a population study, and it is possible, in correlation analyses, to make corrections for the imprecision resulting from measurement variability [17, 18]. This approach is shown in Fig. 1. Figure 1a shows the correlation coefficients in the Gooding study between fasting insulin concentrations and measures of obesity, blood pressure and lipids, as well as a number of other variables that have been proposed to associate with insulin

resistance—NEFAs [19], albumin excretion rate [15], plasminogen activator inhibitor-1 (PAI-1, an inhibitor of fibrinolysis) [20] and markers of low-grade inflammation [16]. The strengths of the associations are shown by the proximity of the variable to the centre. In Fig. 1b, the associations are shown again, but this time after correcting the correlations for variability, calculated as a coefficient of variation for repeated measures over 3.5 years. All the relationships become stronger, in part because the variability of the insulin measurement is nullified, but more notable is the change in the ranking order of variables after such a correction. Thus the corrected correlation coefficients of between 0.25 and 0.30 are seen not only for blood pressure and HDL-cholesterol, but also for C-reactive protein (CRP) and fibrinogen; albumin excretion rate shows a corrected correlation coefficient with hyperinsulinaemia of 0.40; and PAI-1 activity shares with triacylglycerol concentration a corrected coefficient of about 0.7. It seems, then, that the variables that comprise the metabolic syndrome need to be expanded to include PAI-1, markers of low-grade inflammation and microalbuminuria, this last perhaps representing a manifestation of endothelial damage or dysfunction [21, 22]. The issue of biological plausibility of a cause-and-consequence relationship with insulin resistance [23] will be considered below.

Besides issues of defining what is meant by association, there are challenges to the concept of a single cluster. More interrogative approaches to exploring clustering of metabolic syndrome variables are now widely employed, and in particular the technique of factor analysis. Most such studies seem to suggest the existence of three or four separate clusters, generally relating to insulin, to lipids and to blood pressure, with obesity sometimes linked to insulin or lipids, or sometimes forming a separate factor [24–26]. Quite clearly, from these analyses, the concept of a single syndrome revolving around, and caused by, insulin resis-

Fig. 1 Correlations between components of the ‘expanded syndrome X’ and insulin concentrations in the Gooding study [13, 14]. **a** Shows raw correlation coefficients and **b** shows correlation coefficients adjusted for biological and measurement variability. The lengths of the spokes are related to the strengths of the correlations, such that the most closely correlated variables lie closer to the centre. Numbers of measurements for the variables ranged from 107 to 1,034. For further details, see text. *DBP* Diastolic blood pressure, *SBP* systolic blood pressure, *TG* triacylglycerol

tance and/or hyperinsulinaemia is difficult to sustain. And as the metabolic syndrome has expanded to include new members, so has the concept of this single central determinant become even more tenuous.

New members of the metabolic syndrome and their pathophysiology

The next question that will be tackled is that of plausibility of cause-and-consequence relationships with insulin resistance. In his 1988 paper, Reaven proposed convincing mechanisms as to how insulin resistance, in adipose tissue and in the liver, might lead not only to glucose intolerance, but also to the typical metabolic syndrome dyslipidaemia [1]. The mechanisms suggested for the existence of hypertension as part of the syndrome, however, depended more on the hyperinsulinaemia necessary, in an insulin-resistant individual, to maintain euglycaemia [1]. The gold standard for measurement of insulin resistance is

the euglycaemic–hyperinsulinaemic clamp [27], a measure of peripheral glucose uptake under situations of elevated insulin concentrations, a method which is difficult to apply

Hyperinsulinaemia and the metabolic syndrome — issues of plausibility

- Most epidemiological studies of the metabolic syndrome estimate insulin resistance from fasting insulin concentrations (or HOMA-model derived insulin sensitivity)
- Such a measure correlates weakly with clamp insulin sensitivity measures
- Cross-reaction with proinsulin is not a problem with new assays, but proinsulin correlations are generally stronger than those for insulin
- All these considerations raise questions around the plausibility of insulin resistance as a central mechanism

in larger populations studies. For this reason, most such studies use a measure of fasting insulin as a surrogate for insulin resistance, or derive an estimate of insulin resistance using the homeostasis model assessment (HOMA) model [28], which, in non-diabetic populations correlates extremely closely with fasting insulin concentrations, with correlation coefficients of about $r=0.98$ – 0.99 . Other estimates of insulin resistance that are based on a combination of fasting and post-load insulin concentrations can also be used [29, 30]. The relationship between insulin concentrations and clamp measures of insulin resistance is only moderate, with a correlation coefficient of $r=-0.34$ between M value corrected for lean body mass and fasting insulin concentrations in 1,519 subjects in the European Group on Insulin Resistance Database (E. Ferrannini, unpublished data; [31]). This observation has implications for considerations of pathophysiology, for if a variable is regulated by insulin resistance but the measure being employed to assess it is such a poor indicator of insulin resistance, then one might have to postulate other explanations for the associations.

Some years ago, it was shown that the standard insulin immunoassays cross-reacted with the biologically less active insulin precursor molecules proinsulin and its split products [32]. This observation, and emerging assays that permitted specific measures of both insulin and these proinsulin-like molecules, led to studies that found that the correlations of concentrations of these molecules were stronger than those for insulin itself [33], despite their having little biological activity. So once again, epidemiological correlations which were demonstrated were difficult to explain physiologically.

The expansion of the metabolic syndrome to incorporate a series of new candidates has already been introduced.

Thus elevated concentrations or activity of PAI, an inhibitor of fibrinolysis, are regularly described in obese or insulin-resistant subjects [20], as are increased urinary excretion of albumin [15] and indices of low-grade inflammation, such as fibrinogen and C-reactive protein (CRP) [14, 16]. In 1965 Sir Austin Bradford Hill analysed the aspects of epidemiological associations that should be considered before deciding that the most likely interpretation is that of causation—one of which is ‘plausibility’ [23]. And, while recognising that what may, or may not, appear plausible is dependent on prior beliefs [34], in this instance it requires the need for a biological understanding, not only as to why concentrations of proinsulin relate to the metabolic syndrome variables more closely than do those of insulin, but also of how insulin resistance or hyperinsulinaemia might produce inflammation (CRP, fibrinogen) and endothelial dysfunction (microalbuminuria). The issue of confounding is relevant to this argument, as it will be suggested that unconsidered confounders may profoundly influence the relationships observed between variables, either to strengthen or to weaken the apparent correlation.

Associations may be the consequence of unconsidered confounders

Many of the variables that have been related to insulin concentrations or resistance, both original and new, are strongly associated with obesity. As acknowledged by Reaven [35], obesity is a cause of insulin resistance, but is also strongly associated with hypertension, dyslipidaemia and glucose intolerance. Table 2 shows the correlations of BMI with fasting and 2 h insulin concentrations and with the

Table 2 Correlations of metabolic syndrome variables in the Gooding study [13, 14] without and with correction for BMI ($n=1034$)

	Fasting insulin concentration	2 h insulin concentration	Systolic blood pressure	Diastolic blood pressure	Triacylglycerol	HDL-cholesterol
BMI	0.37***	0.31***	0.29***	0.27***	0.35***	−0.30***
Fasting insulin concentration	—	0.51***/0.46***	0.21***/0.11***	0.17***/0.08*	0.30***/0.19***	−0.22***/−0.12***
2 h insulin concentration		—	0.21***/0.13**	0.18***/0.11**	0.30***/0.22***	−0.18***/−0.09**
Systolic blood pressure			—	0.73***/0.71***	0.24***/0.15***	−0.04 ^a /−0.05 ^a
Diastolic blood pressure				—	0.20***/0.12***	−0.07*/0.01 ^a
Triacylglycerol					—	−0.48***/−0.42***

* $p<0.05$

** $p<0.01$

*** $p<0.001$

^a Non-significant correlation

metabolic syndrome variables shown in Table 1, demonstrating the effect of controlling these univariate correlations for BMI. The strength of all relationships is reduced by between one-third and one-half, an effect that might be even greater with a more precise measure of total body fat [36]. But not all fat is alike. Waist circumference, or waist-to-hip ratio, provides a much more powerful prediction of incident cardiovascular disease than does BMI [37], and central fat also contributes strongly to the risk of incident diabetes [38]. Similarly, truncal fat as measured by waist circumference or subscapular skinfold thickness, or visceral fat by computed tomography or magnetic resonance imaging scan, are powerfully related to measures of insulin resistance [39, 40]. Indeed, all three of the widely accepted definitions of the metabolic syndrome now include a measure of central obesity [5–7]. The issue here, then, is whether accurate measures of body fat mass and its distribution might underlie the clustering of risk factors, and if so, whether such an association is the consequence of adipose-induced insulin resistance or through the mediation of some other signal.

As well as unconsidered confounders being responsible for associations attributed to insulin resistance, there is also the possibility that they may help explain relationships which seem biologically implausible. As has been outlined,

Insulin resistance, the metabolic syndrome and endothelial dysfunction—cause, consequence or common antecedent?

- Insulin resistance/hyper/insulinaemia *causes* hypertension, dyslipidaemia, glucose intolerance and endothelial dysfunction (for example [1])
- Endothelial dysfunction *causes* hypertension, glucose intolerance and insulin resistance (for example [45])
- Both endothelial dysfunction and the metabolic syndrome, including insulin resistance, are the consequence of a common antecedent, such as low-grade inflammation [16]

there is an issue of plausibility when the central mechanism proposed to hold together the growing range of metabolic syndrome variables is insulin resistance. In particular, the coexistence of microalbuminuria with insulin resistance in diabetic [41] and non-diabetic subjects [15], and the link of insulin resistance with other measures of endothelial dysfunction [42–44], are difficult to conceptualise as a cause-and-consequence relationship. Although it is possible that there is a reverse aetiology, whereby insulin resistance is a *consequence* of endothelial dysfunction [45], for example through impairment of insulin-dependent vasodilatation [46, 47], of trans-endothelial insulin transport [48]

or of insulin-induced recruitment of nutritive capillary networks [47], another explanation is also possible. This is that *both* insulin resistance and endothelial dysfunction are consequences of a common aetiological mechanism.

Exploring the relationships of metabolic syndrome variables in a population of healthy adults, we reported good correlations with a factor score of low-grade inflammation, derived from circulating concentrations of the cytokines TNF- α and IL-6, and the acute-phase markers CRP and fibrinogen [16]. Indeed, this correlation between an ‘insulin resistance’ z-score and an ‘acute-phase’ z-score of $r=0.59$, suggests that even using these crude approaches, some 35% of the variance of the measures comprising the metabolic syndrome could be ‘explained’ by a state of low-grade inflammation. Furthermore, this ‘acute-phase’ marker score also correlated well with markers of endothelial activation, such that there was no residual statistical link between endothelial dysfunction and insulin resistance once the inflammatory markers were included in the equation [16]. These findings raise the possibility that the association between insulin resistance and the components of the metabolic syndrome is the consequence of their being common outcomes of a state of low-grade inflammation.

A similar conclusion emerges from studies using factor analysis. As outlined earlier, this approach has been used to characterise patterns of clustering among the syndrome X variables, and most such studies have employed only the classic components of the syndrome, plus obesity. If, however, one includes inflammatory markers in such a factor analysis, as we have done in 469 men with myocardial infarction and in 575 controls [49], then the ‘metabolic syndrome’ factor is closely related to a low-grade inflammation factor in both subject groups. Another study, in 236 native Canadian children [50], showed that without inclusion of acute-phase markers, there were relationships of insulin concentrations with dyslipidaemia and obesity, but not with blood pressure. If, however, measures of three acute-phase markers (CRP, IL-6 and serum amyloid A) were included in the model, the relationship between insulin concentrations and dyslipidaemia also disappeared.

The next issue, then, is how to explain a low-grade inflammatory state in healthy subjects. Our own observations failed to find major relationships of these acute-phase markers and cytokines with titres of antibodies against *Helicobacter pylori*, *Chlamydia* and cytomegalovirus [16]. There were, however, strong and consistent relationships with anthropometric measures of obesity and central fat distribution, such that about 20% of the variance of the acute phase z-score could be explained, statistically, on the basis of adiposity [16]. Adipose tissue generates proinflammatory cytokines, whether from adipocytes or from infiltrating macrophages, an observation that has become a

Fig. 2 An inflammation-centred paradigm for 'expanded syndrome X'. *H/T* Hypertension, *TG* triacylglycerol

major research theme in recent years. The pioneering work of Hotamisligil and Spiegelman [51], showing the effect of $\text{TNF-}\alpha$ on inhibition of the insulin signalling pathway, has led to the exploration and characterisation of several other adipocytokines with similar metabolic functions [52]. And the fact that these cytokines inhibit the effect of insulin on endothelial cells [53, 54] may additionally contribute to insulin resistance by limiting nutrient-induced increases in nutritive capillary flow to muscle, as well as muscle glucose uptake.

So a new, and perhaps more likely, paradigm for the metabolic syndrome seems to be that of adipose tissue-generated molecules initiating a state of low-grade inflammation, with the known actions of these proinflammatory cytokines [16] resulting in the combined metabolic, haemodynamic and vascular consequences of this state. And in this model, insulin resistance becomes merely another consequence of this low-grade inflammatory state. The paradigm is illustrated in Fig. 2. But the proposed model raises a new question: How does excess fat mass signal to the organs involved in the pathophysiology of the metabolic syndrome—the liver, skeletal muscle and blood vessels?

The metabolic syndrome—a consequence of endocrine signals?

In large population studies, blood samples are generally taken from a vein, usually in the fasting state. This means that non-endocrine signals, such as those limited to a portal or an arterial circulation, or signals in the postprandial state, remain undocumented.

As outlined above, the best characterised of the adipocytokines, in terms of effects on insulin action, is $\text{TNF-}\alpha$, which has powerful effects on inhibiting insulin signalling in the adipocyte, liver and skeletal muscle, as well as in the whole organism [51, 55]. Moreover, $\text{TNF-}\alpha$ also inhibits endothelial nitric oxide (NO)-dependent vasodilatation [56] and activates production of adhesion molecules by endothelial cells [57]. But $\text{TNF-}\alpha$ does not have the characteristics of a circulating endocrine signal. First, our group has found no net adipose tissue release of the cytokine, as measured by arterio-venous difference across a subcutaneous adipose tissue bed [58]. Second, other than in states of sepsis, circulating concentrations of $\text{TNF-}\alpha$ are below those likely to saturate soluble binding proteins [51]. Is it possible, then, that $\text{TNF-}\alpha$ is acting in a non-endocrine fashion?

A substantial component of adipose tissue $\text{TNF-}\alpha$ production arises from infiltrating macrophages [59], and this infiltration may represent an important paracrine mechanism whereby increasing fat mass induces adipocyte insulin resistance. Increasing fat mass and adipose tissue macrophage infiltration are also associated with increased liver fat content [60], a state in which liver production of $\text{TNF-}\alpha$ is increased and hepatic insulin action impaired [61], again through local and not systemic effects of the cytokine. Ectopic fat within skeletal muscle fibres is also associated with insulin resistance [62]. We have recently suggested that, in a similar fashion, ectopic fat around blood vessels, through increased production and local action of adipocytokines, may also play an important role

Fig. 3 Vasocrine signalling from perivascular fat. Adipocytokines secreted from perivascular adipocytes inhibit the phosphoinositide 3-kinase (*PI3-K*) pathway of insulin signalling, leaving unopposed vasoconstrictor effects of endothelin-1 (*ET-1*). High concentrations of $\text{TNF-}\alpha$ access the vascular lumen, resulting in inhibition of endothelial *PI3-K* pathway insulin signalling in downstream vessels. Reduced insulin-mediated enhancement of muscle nutritive blood flow will contribute to insulin resistance. *EC* Endothelial cell, *eNOS* endothelial nitric oxide synthase, *ERK* extracellular signal-related kinase, *VSMC* vascular smooth muscle cell

in skeletal muscle blood flow and insulin action, and indeed in vascular disease [63].

The endothelium is an insulin responsive organ, with insulin stimulating both vasodilatory and vasoconstrictive pathways. Insulin stimulates NO synthase production and activation through the phosphoinositol 3-kinase pathway, but also activates release of endothelin-1 through the extracellular-regulated kinase 1/2 pathway. In nutritive arterioles supplying skeletal muscle, the vasodilatory pathway predominates, with physiological elevation of insulin concentrations producing rapid shunting of blood flow from non-nutritive circuits, contributing to insulin action through increasing insulin and substrate delivery to insulin-sensitive tissues [64]. Our data from Zucker fatty rats, and from rats fed a high-fat diet, show the development of a cuff of fat around the origin of muscle nutritive arterioles, associated with complete inhibition of insulin-mediated vasodilatation in the downstream vessel, insulin instead producing endothelin-1-mediated vasoconstriction of the arteriole [63]. The fact that the vasoactive effect is downstream from the fat pad has led to the proposal of a ‘vasocrine’ hypothesis [63] (Fig. 3)—that in obesity, signalling molecules such as TNF- α , generated by periarteriolar fat, influence vasoresponsiveness to insulin in the downstream vascular bed fed by the nutritive arteriole, so contributing to the insulin insensitivity. However, because this signal becomes diluted in the general venous, or systemic, circulation it is not sufficient in amount to influence systemic vascular function.

We have also proposed a homology between periarteriolar fat, having a vasoregulatory function in obesity, with other depots of perivascular fat around arteries, in the mediastinum, neck and trunk [63]. Mazurek et al. [65] have shown marked inflammatory cell infiltration in epicardial fat, these cells converting the adipose tissue to a phenotype with high expression and secretion of chemokines and adipocytokines, with probably outside-to-inside signalling effects on arterial plaque inflammation. We have postulated that the relationship between central obesity and coronary heart disease may relate to the homology of visceral and truncal fat on the one hand with periarterial (atherogenic) and periarteriolar fat (insulin resistance) on the other [63].

Demonstrating causation—the need for experiment

The demonstration of correlation is clearly not proof of causation. One of the considerations listed by Bradford Hill [23] as strengthening the presumption of a cause-and-consequence relationship was ‘experiment’—if a putative causal variable is altered and the supposed dependent

variable changes in consequence, then the hypothesis of causation is supported, or at least not refuted [66, 67]. So how good is the evidence on insulin resistance and its relationships with the metabolic syndrome? Is there evidence to implicate low-grade inflammation as the cause of obesity-related insulin resistance? And can insulin resistance or inflammation be implicated in the putative downstream consequence of the metabolic syndrome, cardiovascular disease? A variety of animal studies, in which inflammatory pathways are manipulated in adipose tissue or in macrophages, strongly support the role of inflammation as a cause of insulin resistance [68–70]. But animal studies are not able readily to answer questions about cardiovascular disease. Human studies of organ-specific gene knockouts or upregulation present different obstacles, although the concept of ‘Mendelian randomisation’ has been used, particularly in cardiovascular risk, to explore the role of several putative mechanisms [71]. Human intervention studies are generally pharmacological.

Perhaps, despite everything that has gone before, the most important observation in regard to the role of insulin resistance is the result of the metformin component of the UK Prospective Diabetes Study (UKPDS) [72]. Metformin increases insulin sensitivity, and has effects on lipids and blood pressure [73]. In the UKPDS, overweight participants with type 2 diabetes were randomised to a variety of glucose-lowering agents, including metformin. Of all the interventions, it was only metformin treatment that was associated with a reduction in the incidence of myocardial infarction, by 39% [72]. Perhaps, after all, insulin resistance does play a role in macrovascular disease in diabetic subjects. But what about the ‘insulin sensitisers’—the thiazolidinediones? The first published outcome study using such drugs was the PROactive study [74], which had the headline result of a significant reduction in macrovascular events during treatment with pioglitazone. This study has been heavily criticised on the basis of its failure, in the statistical analysis for this secondary endpoint, to allow for multiple comparisons [75, 76], the primary endpoint being reduced by an insignificant 10%. In any event, in the context of the Bradford Hill criteria [23], the thiazolidinediones might be considered a less valid test of the insulin resistance hypothesis than is metformin, because of the multiple additional effects of this class of drugs on inflammatory and other signalling pathways and on risk factors [77], and not solely on improving insulin signalling. As to the effects of targeting inflammation, there are ongoing studies of salsalate, a derivative of salicylic acid, on incidence of cardiovascular disease in diabetic patients, encouraged by observations that the drug, when used in anti-inflammatory doses, improves insulin sensitivity [78].

Conclusion

This review has attempted to suggest a novel paradigm for the aetiology of the metabolic syndrome and its link to cardiovascular disease. It has also pointed out a number of pitfalls of the epidemiological approach, which, it is suggested, is valuable for generating a hypothesis but is not able rigorously to test it. These pitfalls of epidemiology

The pitfalls of epidemiological approaches to the metabolic syndrome

- Crude biological measures may represent a variety of regulated pathways (for example, blood pressure)
- Assay measures may represent active and inactive molecules (for example, insulin, adiponectin)
- Systemic venous fasting samples are unable to reflect
 - postprandial or diurnal systemic signals
 - regional (for example, arterial, portal or vasocrine) signals
- Measures of association used to impute causation
 - arbitrariness of cut-offs for significance
 - dependence on sample size
 - effect of biological and assay variability
- Single cluster may in fact represent multiple overlapping clusters
- Unconsidered confounders may weaken observed associations
- Unconsidered confounders may be responsible for observed associations
- Associations may represent cause, consequence, or common antecedent
- Testing of causality requires 'experimentation'

are listed in the text box. They include: the crude biological measures that are generally able to reflect only a single component of a highly regulated biological variable; imprecision of assays and naive physiological models; the use of venous systemic fasting samples; simplistic statistical approaches, the significance of the results depending on a variety of factors including sample size and biological variability of the measure; the problem of confounders, whose inclusion and measurement will depend on dominant paradigms; and, without clinical trials, an inability to test causation. In reality an organism is a much more complex series of integrated systems that need more complex approaches to investigate disease mechanisms, involving cell, organ, whole organism and population investigations. As to the mechanisms involved in the clustering of cardiovascular risk factors, it appears that several of these do cluster together in obese individuals,

who demonstrate a state of low-grade inflammation, endothelial dysfunction and insulin resistance. This article has suggested that central distribution of fat, and its ectopic deposition in liver, muscle and around blood vessels, may be the unifying mechanism linking central obesity, insulin resistance and macrovascular disease. But like any scientific conjecture, this is now merely available for refutation [66].

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Duality of interest The author has no duality of interest.

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