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REVIEW

# Novel biochemical risk factors for type 2 diabetes: pathogenic insights or prediction possibilities?

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**Abstract** This review critically appraises studies examining the association of novel factors with diabetes. We show that many of the most studied novel and apparently 'independent' risk factors are correlated with each other by virtue of their common origins or pathways, and that residual confounding is likely. Available studies also have other limitations, including differences in methodology or inadequate statistical analyses. Furthermore, although most relevant work in this area has focused on improving our understanding of the pathogenesis of diabetes, association studies in isolation cannot prove causality; intervention studies with specific agents (if available) are required, and genetic studies may help. With respect to the potential value of novel risk factors for diabetes risk prediction, we illustrate why this work is very much in its infancy and currently not guaranteed to reach clinical utility. Indeed, the existence of several more easily measured powerful predictors of diabetes, suggests that the additional value of novel markers may be limited. Nevertheless, several suggestions to improve relevant research are given. Finally, we show that several risk factors for diabetes are only weakly associated with the risk of incident vascular events, an observation that highlights the limitations of attempting to devise unified criteria (e.g. metabolic syndrome) to identify individuals at risk of both CHD and diabetes.

**Keywords** Adipose tissue · Association · Biomarker · Causality · Ectopic fat · Endothelium · Hepatic Fat · Inflammation · Prediction

**Abbreviations**

| | |
|---|---|
| ALT | alanine aminotransferase |
| ARIC | Atherosclerosis in Communities |
| BRHS | British Regional Heart Study |
| CAM | cell adhesion molecule |
| CRP | C-reactive protein |
| GGT | γ-glutamyl transferase |
| HOMA | homeostasis model assessment |
| ICAM-1 | intercellular adhesion molecule 1 |
| IRAS | Insulin Resistance Atherosclerosis Study |
| NAFLD | non-alcoholic fatty liver disease |
| PAI-1 | plasminogen activator-1 |
| ROC-AUC | area under the receiver-operating characteristic curve |
| SHBG | sex hormone-binding globulin |
| t-PA | tissue plasminogen activator |
| VCAM-1 | vascular cell adhesion molecule 1 |
| vWF | von Willebrand factor |
| WOSCOPS | West of Scotland Coronary Prevention Study |

## Introduction

In part fuelled by rising rates of diabetes worldwide, interest in 'novel predictors' and pathways for type 2 diabetes is mounting. This review examines evidence for associations between novel biochemical markers (biomarkers) and the risk of type 2 diabetes. We illustrate pathophysiological links be-

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tween novel biomarkers, and show that many reflect processes related to insulin action or resistance that are mediated through the location and function of fat, including excess hepatic fat, or via related inflammation or endothelial dysfunction. In so doing, we highlight multiple correlations between relevant biomarkers; consequently, the extent to which many biomarkers provide additional information about the risk for future diabetes or insights into pathogenic pathogenesis has been overstated. It is acknowledged that some novel risk factor data have contributed to new pathogenic insights, and potential new therapies for diabetes—particularly in the area of inflammatory suppression—are being tested. Whilst the majority of research on novel risk factors seems to be justifiably directed at better understanding pathogenesis, we appraise whether any of the biomarkers can help identify those at high risk of type 2 diabetes, and, if not, speculate on what further work is needed to advance this line of investigation. However, before considering the evidence linking novel biomarkers to diabetes, some general considerations on study design, data interpretation, statistical methods (e.g. difference between association and prediction), residual confounding and related issues are reviewed. In addition, we consider how well the known risk factors, assessed by simple questions or routinely available tests, predict diabetes.

## General comments on the design and interpretation of diabetes novel risk factor studies

1. Based on relative risk estimates in the form of hazard ratios, odds ratios or rate ratios, the usual conclusions of association studies are that an 'independent' association exists between the marker and type 2 diabetes. Such conclusions are however dependent on the assumption that the multivariable models in such studies account for all the important confounders and mediators, which is often not the case, since other relevant variables arising from the same source are often overlooked. Thus, residual confounding is common (see text box). Conversely, some studies adjust for factors likely to be on the causal pathway (e.g. insulin resistance measures), potentially resulting in over-adjustment. Regardless of this, associations, even those of high magnitude, are not synonymous with causality or clinical utility.
2. Analytical measurement error or biological variation in novel biomarkers has largely been neglected in prospective diabetes studies. To overcome this, repeated measurements of biomarkers and correction for regression dilution bias is needed. Such work is now common in vascular literature [1, 2].
3. The association of some novel biomarkers with incident diabetes may vary by age, adiposity (e.g. adiponectin [3]), sex (e.g. C-reactive protein [CRP] [4]) and ethnicity; such factors require greater attention.
4. Statistical methods have varied considerably, with some studies reporting associations in tertiles, quartiles or quintiles of baseline biomarkers or, indeed, arbitrary cut-off values, and other studies using continuous variables. Comparisons across studies are therefore difficult.
5. Given the likely differential contributions over time of certain pathways/organs to the pathogenesis of diabetes, there may be distinct patterns of early vs late predictors of diabetes.
6. The positive effects of lifestyle or drugs on novel risk predictors/pathways are often used to enhance credibility of a causal association. For example, lifestyle improvements, metformin, and glitazones all lower CRP and plasminogen activator-1 (PAI-1) levels [5–8], and such findings are used to reinforce a causal role for inflammation or fibrinolysis in the pathogenesis of diabetes. However, caution against such interpretations is needed, since all such interventions simultaneously influence other pathways relevant to diabetes.
7. As regards outcome variables, diabetes has been diagnosed by different methods: studies have used either self-report or new prescriptions, but few have had detailed biochemical confirmation. In addition, studies have varied markedly in their rigour to exclude participants with prevalent diabetes, again making comparisons challenging. More robust and standardised methods would advance this field.

*Prediction vs association* Much of the relevant research has been justifiably targeted towards improving our understanding of the pathogenesis of diabetes. The possibility that novel biomarkers could serve as a screening tool for predicting future diabetes, and hence play a part in its prevention, has been liberally raised by some authors, thereby confusing the otherwise separate issues of aetiology and prediction. The additional utility of a new biomarker test for risk prediction should be assessed on the test's performance (e.g. sensitivity, specificity, positive predictive value) in the context of existing predictors, using tools such as statistical models that compare the areas under the receiver-operating characteristic curves (ROC-AUCs) or C-statistics for risk scores calculated without and with the novel risk factor. However, in diabetes, such systematic assessment using appropriate statistical approaches is generally lacking.

*Established 'predictors' of type 2 diabetes: how good are they?* Before appraising the role of novel diabetes biomarkers, we should consider how good known risk factors are at predicting diabetes. Obesity is a major risk factor for type 2 diabetes, and BMI, waist circumference and waist/hip ratio predict incident diabetes with ROC-AUCs ranging from 0.66 to 0.73 [9]. Similarly, glycaemia is a strong predictor of future diabetes risk, with AUCs ranging from

**Key limitations of current research on novel biomarkers for diabetes, and suggested improvements**

| Area | Limitations | Possible solutions |
|---|---|---|
| Residual confounding | Residual confounding is common: many parameters examined in isolation have some overlap of origin with other proposed novel risk markers so that levels correlate | More comprehensive studies examining a range of parameters from common sources |
| Measurement variation | Consideration of analytical/biological variation limited | Repeat measures in a subgroup may be helpful to correct for regression dilution, and over longer periods could be used to assess dynamic changes |
| Statistics | Statistical methods and reporting of results vary tremendously, making comparisons difficult | Standardisation of reporting. All studies should consider reporting associations with a 1 SD change in a novel parameter, if appropriate |
| Prediction vs association | Lack of studies examining extent of prediction afforded by novel risk factors beyond known predictors or simple algorithms to detect individuals at high diabetes risk | More use of ROC analyses, C statistics or similar statistical methods to place results into meaningful clinical context. There is no guarantee that novel risk factors will improve risk prediction |
| Differing populations | Associations of risk factors with incident diabetes will vary according to age, sex and ethnicity—more research needed | More studies in different population subgroups needed |
| Diabetes vs vascular risk markers | Risk factors for diabetes may not necessarily predict vascular events, or do so with equal strength | Further research comparing and contrasting risk factors for diabetes and vascular events is needed |
| Causal inferences | Association studies in isolation cannot prove causality, regardless of their robustness or comprehensiveness | Appraisal against criteria for causality, and combination of biomarkers with genetic polymorphisms (Mendelian randomisation) and, where possible, interventions with specific agents, are needed to advance claims of causality |

0.73 to 0.77 for fasting or post-load glucose measures [10]. Combinations of obesity and easily available biochemical/clinical measures have reasonable predictive ability for diabetes, perhaps better than an OGTT. In a Swedish study, a combination of $HbA_{1c}$, fasting glucose and BMI achieved a specificity of between 93% and 97% and a sensitivity of between 52% and 66%, with comparable results on addition of a positive family history, OGTT or triacylglycerol [11]. Similarly, a multivariable model with readily available clinical variables (age, sex, BMI, ethnicity, family history, systolic blood pressure, fasting glucose and HDL-cholesterol) achieved a greater AUC (0.84) than the 2 h glucose value alone (AUC 0.78), while addition of 2 h glucose measurement to the clinical model increased the AUC modestly from 0.84 to 0.86, thus not justifying its greater cost and inconvenience [12]. More recently, a simple clinical model including family history of diabetes, obesity, blood pressure, lipids and impaired fasting glucose produced an AUC of 0.85 for prediction of incident diabetes in the Framingham Offspring Study [13]. Notably, more complex clinical models that included OGTT, fasting insulin, and C-reactive protein levels or homeostasis model assessment (HOMA) indexes of insulin sensitivity and beta cell sensitivity did not improve this AUC [13].

*Use of risk scores or questionnaires for diabetes risk prediction* As a further development, the concept of 'risk scores', calculated using routinely available or easily collectable data, has emerged as an appealing tool for predicting both undiagnosed prevalent diabetes and the risk of future incident diabetes. The Cambridge Diabetes Risk Score uses general practice record data (age, sex, BMI, history of antihypertensive or steroid medication, family history and smoking history) to give a reasonable prediction of prevalent undiagnosed diabetes (AUC 0.80) [14]. Using categorical variables for age, BMI, waist circumference, history of antihypertensive drugs or high blood glucose, physical activity and daily consumption of fruit/berries/vegetables, the Finnish Diabetes Risk Score achieved an AUC of between 0.85 and 0.87 for 10 year incident diabetes [15]. The recent German Diabetes Risk Score, comprising age, height, waist circumference, history of hypertension, physical activity, smoking and dietary factors,

has reported an AUC for incident diabetes of between 0.82 and 0.84 [16]. It is important to appraise whether novel risk markers can increase risk prediction beyond such simple risk scores.

*Challenging benchmarks for novel biomarkers* As discussed above, any added value of novel biomarkers might be limited. Parallels from the situation in cardiovascular risk prediction are relevant. Analyses from the Framingham Heart Study and the Atherosclerosis in Communities (ARIC) study failed to show significant incremental usefulness of ten and 19 novel biomarkers, respectively, for predicting cardiovascular risk beyond conventional risk factors [17, 18].

Having considered the criteria against which we might appraise any 'new kids on the block', we now proceed to examine specific novel biomarkers.

## Categorising novel predictors of type 2 diabetes

We subdivide putative predictors into those derived from adipose tissue, the liver or the endothelium; those arising from several sources (e.g. PAI-1) are noted in the text. The text box 'Novel biochemical predictors of type 2 diabetes—narrative review of strengths of association with incident diabetes, adjustments made, correlated factors and caveats' provides a summary of representative data. As inflammatory markers arise from immune cells and from all of the above tissues, they are discussed separately. Figure 1 summarises the major origins of the parameters discussed. We review those parameters for which most data exist, rather than discussing all circulating blood parameters previously linked to incident diabetes, which is beyond the scope of this article.

## Adipose-derived predictors of type 2 diabetes

*Adiponectin* Adiponectin differs from the other adipocyte hormones in that its concentrations decline with increasing obesity. Besides inhibiting inflammatory pathways, recombinant adiponectin increases insulin sensitivity and enhances lipid clearance. Its insulin-sensitising effect is largely attributable to suppression of hepatic glucose production, but beneficial effects on muscle also exist. The molecular actions of adiponectin have recently been reviewed [19, 20].

An inverse association between adiponectin and the risk of incident type 2 diabetes is consistent across diverse populations [3, 21–24]. In the British Regional Heart Study (BRHS), men with adiponectin concentrations in the top tertile (vs the bottom tertile) had a 60% (95% CI 30–77%) lower risk of incident type 2 diabetes after 5 years (adjustments made are detailed in the text box) [3]. Further adjustment for insulin resistance attenuated the association. In addition, the link between elevated adiponectin levels and lower risk of diabetes was significantly stronger in obese men than leaner counterparts, and may also be stronger in women [24, 25].

There are complexities in the adiponectin story. For example, the initial suggestion that higher adiponectin predisposes individuals to lower vascular risk [26] has been challenged [2]. High adiponectin levels have been linked to high—not low—rates of all-cause and cardiovascular mortality in prospective general population studies [27, 28]. It is also unclear why adiponectin levels increase with age. Finally, the potential differential role of adiponectin isomers needs to be clarified: compared with plasma total adiponectin, the ratio of the high-molecular-mass isomer to total adiponectin is more strongly correlated with the HOMA index of insulin resistance (HOMA-IR; ROC-AUC 0.61 vs 0.71) [29].

In summary, the role of adiponectin in diabetes risk is of continued interest but far from fully elucidated, and is currently centred on pathogenesis rather than prediction.

*Leptin* Leptin regulates body weight by effects on food intake and metabolism. In healthy humans, leptin is an excellent biochemical marker of percentage fat mass. Leptin has been related to insulin resistance and associated variables, such as triacylglycerol, inflammatory factors, and low HDL-cholesterol, independent of waist circumference. However, the extent to which leptin is independently related to risk of diabetes remains unclear. While one study has indicated a positive association with incident diabetes in Japanese-American men [30], another has suggested no independent relationship between leptin and diabetes [31]. In the BRHS, leptin (top vs bottom tertile) was associated with a relative risk of diabetes of 1.91 (95% CI 0.97–3.76) in analyses adjusting for several confounders, including waist circumference, but further adjustment for insulin resistance abolished the association (adjusted RR 1.12, 95% CI 0.55–2.26) [3]. In the ARIC study, high leptin levels were associated with an increased risk of diabetes (HR 3.9, 95% CI 2.6–5.6), with adjustment only for age, sex, ethnicity and centre. However, upon further adjustment for factors related to leptin resistance (such as obesity, insulin, inflammation score, hypertension, triacylglycerol and adiponectin), an apparent protective association (HR 0.59, 95% CI 0.23–0.67) was noted [32]. At present, therefore, any independent association of leptin with incident diabetes is difficult to establish because of methodological and analytical differences between studies.

*PAI-1* Elevated PAI-1 levels reflect a state of compromised fibrinolysis or an acute phase response. PAI-1 may be synthesised from adipocytes, hepatocytes and endothelial cells, and circulating levels increase with adiposity. Several studies, including the Insulin Resistance Atherosclerosis Study (IRAS), have linked high PAI-1 levels to incident diabetes

**Novel biochemical predictors of type 2 diabetes—narrative review of strengths of association with incident diabetes, adjustments made, correlated factors and caveats**

| Novel predictor | Evidence for association of baseline levels with incident diabetes | Strength of independent association from a representative study or summary meta-analysis[a] | Factors adjusted in analysis | Examples of observed correlations with other relevant parameters | Other points of relevance/ caveats |
|---|---|---|---|---|---|
| **Adipose-derived** | | | | | |
| Adiponectin | Consistent inverse associations independent of obesity, across ethnic groups and sex. Association may be stronger in obese individuals | Top tertile vs bottom tertile RR 0.40 (95% CI 0.23–0.70) [3] | Age, BMI, social class, physical activity, smoking status, alcohol intake, pre-existing CHD/stroke, statins, systolic BP, treatment for BP | +ve: HDL-C, age, renal dysfunction –ve: obesity, liver fat, ALT, PAI-1, ferritin, triacylglycerol | High adiponectin less strongly associated with lower CHD risk<br>High adiponectin predicts higher all-cause mortality |
| Leptin | Inconsistent | High baseline leptin levels linked to elevated risk, no risk and low risk of incident diabetes [30–32] | Variations in statistical adjustments may partly explain differing results; more data needed | +ve: obesity, CRP, triacylglycerol, PAI-1, ALT –ve: HDL-C | Not a routine test, and thus not standardised; sexual dimorphism, with much higher levels in women |
| IL-6 | Consistent associations independent of obesity and insulin resistance | Top tertile vs bottom tertile 2.02 (95% CI 1.14–3.58) [3] | Age, BMI, social class, physical activity, smoking status, alcohol intake, pre-existing CHD/stroke, statins, systolic BP, treatment for BP | +ve: obesity, CRP, triacylglycerol, PAI-1 –ve: HDL-C, adiponectin | IL-6 associated with higher all-cause mortality and other disease outcomes, i.e. non-specific |
| PAI-1 (also derived from liver, endothelium) | Consistent association with incident diabetes independent of obesity and insulin resistance | 1.61 (95% CI 1.20–2.16) for 1 SD increase [33] | Age, sex, clinical centre, smoking, ethnicity, $S_I$, BMI, family history of diabetes, physical activity | +ve: liver fat, liver enzymes, triacylglycerol –ve: adiponectin, SHBG | Not a routine test and thus not standardised. PAI-1 rise over time is also associated with diabetes, but associations adjusting for LFTs or other markers of liver fat not tested |
| **Liver-derived** | | | | | |
| ALT/GGT | Reasonably consistent associations with incident diabetes independent of obesity and insulin resistance | Top quartile vs bottom quartile ALT 2.72 (95% CI 1.47–5.02) GGT 3.68 (95% CI 1.68-8.04) [42] | Age, social class, physical activity, smoking status, alcohol intake, pre-existing CHD or stroke, use of statins, BMI | +ve: liver fat, triacylglycerol, PAI-1, ferritin –ve: adiponectin | Assays routine and standardised ALT appears to rise more steeply in men who develop diabetes |

| Novel biochemical predictors of type 2 diabetes—narrative review of strengths of association with incident diabetes, adjustments made, correlated factors and caveats | | | | | |
|---|---|---|---|---|---|
| SHBG | Evidence of association consistent | RR 0.20 (95% CI 0.12–0.30) in women (SHBG levels >60 vs ≤60 nmol/l) RR 0.48 (95% CI 0.33–0.69) in men (SHBG levels >28.3 vs ≤28.3 nmol/l) [45] | Variable, but limited number of other hepatic-derived factors | +ve: HDL-C, adiponectin –ve: triacylglycerol, PAI-1, CRP, ferritin | Although routine assays available, SHBG is influenced by sex and, in women, by reproductive stage or exogenous hormonal use |
| Ferritin | Emerging evidence of association | Clincally raised group vs bottom quartile of normal range OR 3.2 (95% CI 1.3–7.6) [48] | Age, BMI, sex, family history, physical activity, smoking habit, dietary factors, CRP, IL-6, fibrinogen, LFTs, adiponectin | +ve: liver fat, obesity, liver enzymes –ve: adiponectin, vitamin C | Routinely measured in clinical practice. Acute-phase reactant |
| IGF-1 | Modest evidence of inverse association | OR 0.50 (95% CI 0.26–0.95) when IGF-1 greater than median [50] | Age, sex, waist, BMI, IGFBP-1, 2 h post-load glucose, 0 h insulin | +ve: HDL-C, adiponectin –ve: triacylglycerol, obesity, insulin resistance | High levels associated with increased risk of cancers |
| CRP | Consistent association with incident diabetes independent of obesity | CRP >2.6 mg/l vs <0.5 mg/l RR 2.37 (95% CI 1.57–3.58) [67] | Age, BMI, various | +ve: obesity, triacylglycerol, IL-6, leptin –ve: HDL-C, adiponectin | Although assays are routine, short-term variability is high CRP may also be less strongly associated with incident vascular events, and is non-specific |
| **Endothelial-derived factors** | | | | | |
| CAMs | Emerging evidence of association independent of obesity, other markers of inflammation, and insulin resistance | Top quintile vs bottom quintile E-selectin RR 5.43 (95% CI 3.47–8.50) ICAM-1 RR 3.56 (95% CI 2.28–5.58) [63] | BMI, family history diabetes, smoking, diet score, alcohol, activity index, post-menopausal hormone use | +ve: lipids, obesity, CRP, IL-6 –ve: HDL-C | Assays not standardised; CAMs do not appear to be independently predictive of vascular events in most studies |
| t-PA antigen | Emerging evidence of association | Top quartile vs bottom quartile OR 6.5 (95% CI 1.3–33) [58] | Age, sex, diastolic BP, waist circumference, insulin, triacylglycerol, fasting and post-load glucose | +ve: triacylglycerol, obesity, –ve: adiponectin | Assays not standardised Associations independent of CAMs, PAI-1 and other liver-derived markers not well studied |

| Novel biochemical predictors of type 2 diabetes—narrative review of strengths of association with incident diabetes, adjustments made, correlated factors and caveats | | | | | |
|---|---|---|---|---|---|
| vWF antigen | Evidence of association | RR per interquartile range increase 1.49 (95% CI 1.21–1.85) [62] | Physical activity, lipids, BP, smoking, family history, alcohol, NSAIDS, oestrogen-containing or BP therapy, IGT, waist circumference, HOMA-IR, CRP | +ve: inflammatory factors, t-PA, IR<br>–ve: HDL-C | No studies simultaneously examining associations of all of vWF, PAI-1 and CAMs with incident diabetes |
| **Nutritional-related markers** | | | | | |
| Vitamin C | Emerging evidence of inverse association in cross-sectional analyses | Limited data on association of plasma vitamin C and incident diabetes | – | +ve: physical activity, alcohol<br>–ve: CRP, SES, smoking, high-fat diet, low-fibre diet | Low vitamin C levels associated with incident CHD events, but causality is questioned |
| Vitamin D | Emerging evidence of inverse association in cross-sectional analyses | Limited data on serum vitamin D and incident diabetes | – | +ve: vitamin supplements, ↑milk and dairy products; favourable dietary profile<br>–ve: smoking, alcohol, and CRP | Ongoing trials of vitamin D in diabetes/glucose intolerance |

[a] Different studies have used different methods (e.g. tertiles, quartiles, 1 SD) to link novel parameters with incident diabetes, and have adjusted for different factors, and so RR or HR should not be compared between parameters

HDL-C, HDL-cholesterol; IGFBP-1, IGF binding protein 1; LFTs, liver function tests; NSAIDs, non-steroidal anti-inflammatory drugs; SES, socioeconomic status

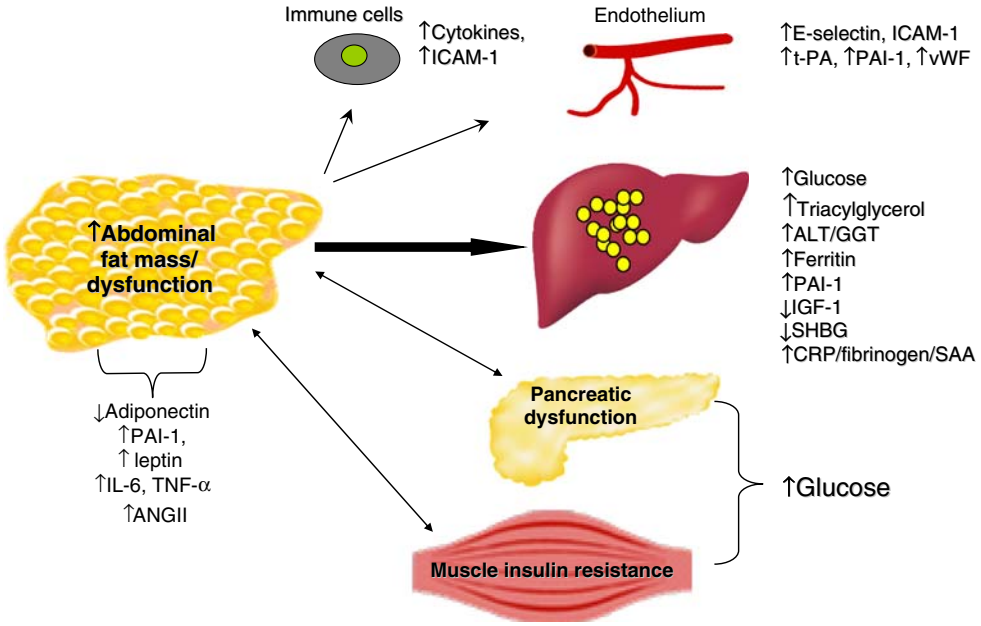


**Fig. 1** Common origins of novel diabetes risk factors. This figure depicts the potential pathophysiological links between many proposed novel predictors of type 2 diabetes and is intended to be more conceptual than comprehensive. Clearly, some novel biomarkers give insights into fat mass/location/dysfunction and thus provide metabolic information that is beyond that provided by simple anthropometric (e.g. BMI, waist circumference) measures. Equally, many other biomarkers variably give insight into hepatic fat accumulation, whilst others reveal dysfunctional immune or endothelial function. Several variables also arise from multiple sources. Although not included, it is important to note that insulin action is relevant to all represented tissues. Insulin has mostly favourable effects (see text), although in some cases the effects of hyperinsulinaemia can be potentially detrimental. ANGII, angiotensin II; SAA, serum amyloid A

[33]. In the IRAS, PAI-1 showed a stronger association than inflammatory factors with incident diabetes. In a logistic regression model (see text box), PAI-1 (but not CRP or fibrinogen) remained significantly related to incident type 2 diabetes (OR for 1 SD increase 1.61, 95% CI 1.20–2.16) [33]. Furthermore, an increase in PAI-1 (but not fibrinogen) levels over time was also associated with incident diabetes [34].

Several cytokines and hormones, including TNF-α, angiotensin II and insulin, positively regulate the expression of the gene encoding PAI-1. Thus, rather than being directly linked to diabetes, PAI-1 may be indirectly related via inflammation or hyperinsulinaemia. The data from IRAS [33], described above, in which adjustments for CRP and insulin were made, would seem to lessen this possibility. However, other data, from the Strong Heart Study, suggest that insulin and insulin resistance partly explain the association of PAI-1 with incident diabetes [35]. Alternatively, an association between high PAI-1 and incident diabetes may reflect common associations with liver fat. PAI-1 levels correlated with baseline liver fat content in patients with highly active antiretroviral therapy-associated lipodystrophy, and were reduced in parallel with reductions in liver fat in response to glitazone therapy in such patients [36]. PAI-1 levels are also raised early in animal models of fatty liver [37].

Although results from animal studies lend support for a causal link between elevations in PAI-1 and diabetes, available genetic data (4G/5G polymorphism) currently indicate that PAI-1 does not play a causal role in diabetes [38].

## Hepatic-derived ‘predictors’

*Alanine aminotransferase and γ-glutamyl transferase* Elevated alanine aminotransferase (ALT) levels within the high-normal range is associated with type 2 diabetes independently of a range of confounding factors, including obesity [39]. Findings from the West of Scotland Coronary Prevention Study (WOSCOPS) [40] are consistent with a number of other studies; compared with men with values for baseline ALT in the bottom quartile (<17 U/l), those with levels in the top quartile (>29 U/l) had an adjusted odds ratio of 2.04 (95% CI 1.16–3.58) for incident diabetes.

Why should elevations in ALT predict diabetes? A likely link is liver fat since elevated ALT levels even within the normal range correlate with increasing liver fat [39]. Indeed, ALT is used to diagnose non-alcoholic fatty liver disease (NAFLD), often in conjunction with liver ultrasound. The

mechanisms linking excess hepatic fat to insulin resistance are now beginning to emerge [41].

In terms of an association with incident diabetes, γ-glutamyl transferase (GGT) is at least as strong as ALT. In the BRHS, the risk of type 2 diabetes increased significantly with increasing levels of ALT and GGT, even after adjustment for a range of confounders, including BMI (top vs bottom quartile, ALT: RR 2.72, 95% CI 1.47–5.02; GGT: RR 3.68, 95% CI 1.68–8.04) or with further additional adjustment for insulin resistance [42]. Of note, liver enzymes correlate with other factors, such as low adiponectin and high PAI-1 (see text box) but data on parallel examination of these parameters in relation to incident diabetes are sparse. Finally, initial results from a meta-analysis suggest that the association of liver enzymes/fatty liver with incident vascular events is likely weaker than the link between liver fat and diabetes [43].

*Sex hormone-binding globulin* Sex hormone-binding globulin (SHBG) is hepatically secreted and the major binding protein for plasma sex steroids, regulating the availability of free steroids for hormone-responsive tissues. Hepatic SHBG production is downregulated by insulin, and low levels reflect insulin resistance [44]. As such, SHBG is correlated with many other factors associated with diabetes (see text box).

In a recent meta-analysis of prospective studies, women with higher SHBG levels (>60 vs >60 nmol/l) had an 80% lower risk of type 2 diabetes (RR 0.20, 95% CI 0.12–0.30), while men with higher SHBG levels (>28.3 vs ≤28.3 nmol/l) had a 52% lower risk (RR 0.48, 95% CI 0.33–0.69) [45]. Thus, low SHBG appears more strongly linked to incident diabetes in women. However, few relevant studies have adjusted for other commonly measured liver-derived parameters (e.g. triacylglycerol, ALT) that are linked to diabetes risk, and therefore more comprehensive studies are needed.

*Ferritin* Serum ferritin is used as an indicator of iron stores, and is considered a nutritional biomarker. However, it is also a positive acute phase reactant. Recent studies indicate a positive association between serum ferritin and risk of incident type 2 diabetes [46–48]. However, a case–cohort study nested within the ARIC cohort reported that adjustment for BMI and components of the metabolic syndrome attenuated, and rendered non-significant (HR 0.81, 95% CI 0.49–1.34), the previously significant association (HR 1.74, 95% CI 1.14–2.65) between ferritin and diabetes [49]. In contrast, in the European Prospective Investigation of Cancer (EPIC)-Norfolk study, the risk of clinically incident diabetes was markedly elevated in participants with clinically raised ferritin compared with those with ferritin levels in the lowest quartile (OR 7.4, 95% CI 3.5–15.4) [48]. It should be noted that fasting glucose was not adjusted for, and might potentially contribute to the high odds ratios observed in this study. Nevertheless, further adjustment for potential confounding by inflammation had no material impact on the observed association, whereas adjustment for hepatic enzymes and adiponectin did (OR attenuated to 3.2, 95% CI 1.3–7.6) [48]. The latter observation is consistent with a potential link between ferritin levels and hepatic fat accumulation. Of course, iron may damage hepatocytes directly, promote oxidative stress and impede insulin suppression of hepatic glucose production. Equally, iron excess may also contribute to decreased insulin secretion. The important concept, once again, is that, because of the multiple inter-relationships between ferritin and other diabetes-related factors, further research is needed to determine whether there is a causal role for elevated iron stores in diabetes, or whether elevated plasma ferritin is simply a metabolic abnormality associated with diabetes development.

*IGF-1* The IGF system plays a key role in somatic growth regulation and organ development in childhood, and in tissue regeneration and metabolic regulation throughout life. Together with insulin, IGF-1 is important in glucose metabolism and homeostasis. Circulating IGF-1 is largely produced in the liver, under the influence of growth hormone. In the Ely study, IGF-1 levels above the median were associated with a lower risk of subsequent impaired glucose tolerance or diabetes (OR 0.50, 95% CI 0.26–0.95) (adjustments detailed in the text box) [50]. Larger studies are needed to confirm this association since, as discussed by Sandhu et al. [51], current evidence linking IGF-1 with diabetes and CHD is susceptible to chance, reverse causality or residual confounding. Indeed, other than its negative association with obesity and insulin resistance, IGF-1 is positively correlated with HDL-cholesterol and is thus likely to show an inverse relationship with triacylglycerol (see text box) so that such factors, and probably others including influence of binding proteins (such as IGF binding protein 2 [52]), need to be co-analysed in future studies.

*Fasting glucose and triacylglycerol as correlates of hepatic fat* Although this article focuses on novel biomarkers, it is important to note that excess liver fat is correlated with hepatic gluconeogenesis, the major contributor to fasting glucose levels [53]. Similarly, fasting triacylglycerol is a reflection of hepatic VLDL synthesis, which is also correlated with hepatic fat content [54]. Thus, overweight individuals who have a combination of slightly elevated fasting glucose and triacylglycerol (and lower HDL cholesterol), plus raised ALT reveal themselves to have excess liver fat. Routinely performed tests therefore give some insight into ectopic hepatic fat accumulation. Of interest,

both fasting glucose and triacylglycerol are more strongly associated with incident diabetes than with CHD risk [55].

## Endothelial-derived parameters

Endothelial dysfunction may play a role in insulin resistance, and the reverse is also likely [56, 57]. Circulating levels of several endothelial-derived factors, cell adhesion molecules (CAMs), tissue plasminogen activator (t-PA; and PAI-1) and von Willebrand factor (vWF), have been linked to type 2 diabetes risk.

*t-PA* Although the vascular endothelium is a source of t-PA, higher plasma t-PA antigen represents largely inactive circulating t-PA–PAI-1 complexes, and these could reflect endothelial disturbance (t-PA release) and/or elevated PAI-1 levels from liver and adipose tissue. Given that PAI-1 is associated with incident diabetes, it is no surprise that elevated t-PA levels may be similarly related, but only one published study to date has reported such data [58]. The northern Sweden Monitoring of Trends and Determinants in Cardiovascular Diseases (MONICA) study reported an increased risk of diabetes (OR 6.5, 95% CI 1.3–33) in individuals with t-PA antigen levels in the highest vs the lowest quartile after adjustment (see text box). The small number ($n$=15) of incident cases in this study explains the wide confidence interval. Like PAI-1, t-PA antigen shows multiple correlations with other insulin resistance factors, and additional studies in which these factors are comprehensively investigated are needed.

*vWF* Levels of vWF antigen in the circulation are interpreted as a reflection of endothelial activation. Data on the relationship between vWF and diabetes risk have been inconsistent. While some studies have reported no association, or no independent association, between vWF and diabetes [59–61], vWF was shown to be predictive of diabetes in the Framingham Offspring Study [62]. The age- and sex-adjusted relative risk of diabetes was 1.49 (95% CI 1.21–1.85) per interquartile range increase in vWF among the participants of the latter study [62]. This effect remained (RR 1.39, 95% CI 1.09–1.77) after further adjustment for a range of factors (see text box). Studies simultaneously examining associations of vWF, PAI-1 and cell adhesion molecules with incident diabetes are sparse.

*CAMs* CAMs such as E-selectin are expressed by the vascular endothelium in response to a variety of toxic stimuli, including inflammation, and their shedding into the systemic circulation is considered to reflect endothelial dysfunction. In the Nurses Health Study, adjusted analyses revealed that the relative risk of incident diabetes for individuals with E-selectin levels in the highest quintile (vs the lowest) was 5.43 (95% CI 3.47–8.50), and the corresponding risk for intercellular adhesion molecule 1 (ICAM-1) was 3.56 (95% CI 2.28–5.58; see text box) [63]. VCAM-1 was not associated with incident diabetes. Adjustment for waist circumference instead of BMI or further adjustment for CRP, fasting insulin and $HbA_{1c}$ did not alter associations [63]. Similar results have been reported by the Women's Health Initiative study [64]. Because E-selectin is expressed exclusively by endothelial cells, whereas ICAM-1 and VCAM-1 are expressed on a number of other cells, E-selectin may be the better marker of early endothelial dysfunction. However, the extent to which CAMs are associated with diabetes risk independently of factors that are easier to test (e.g. blood lipids) has not been well tested. On the basis of the available evidence [e.g. ref. 65], circulating concentrations of CAMs show weaker associations with incident CHD than with incident diabetes.

## Inflammatory factors

A body of literature links inflammatory factors to obesity and type 2 diabetes. Early cross-sectional observations of elevated inflammatory markers in diabetes were quickly followed by prospective studies demonstrating that CRP, IL-6 and white cell count were all independently associated with incident type 2 diabetes. In parallel, and as reviewed by Hotamisligil [66], our understanding of the physical and molecular links between immune function and metabolism has increased. For example, we now know that adipose tissue and liver have an architectural organisation in which metabolic cells (adipocytes or hepatocytes) are in close proximity to immune cells (Kupffer cells or macrophages) [66]. Although the close coordinated regulation of metabolic and immune function is generally advantageous, on the downside, over-nutrition or metabolic stress can lead to aberrant immune responses, and vice versa. Of course, the 'inflammation' in diabetes is not typical of an acute inflammatory response or injury, but, rather, represents a low 'grumbling' chronic inflammation, characterised by the more modest elevations in CRP levels detected by high sensitivity assays.

*CRP* CRP is hepatically derived, IL-6 being the main stimulus for its production. CRP has consistently been associated with incident diabetes, as summarised in a recent publication [67]. According to this meta-analysis of nine studies, individuals with high CRP levels (>2.6 mg/l) had a relative risk of diabetes of 2.37 (95% CI 1.57–3.58) compared with those with a low CRP level (<0.5 mg/l), after adjusting for obesity [67].

CRP is correlated with several other parameters relevant to diabetes, including lipids, SHBG and adiponectin, but few studies to date have assessed all relevant markers in parallel. CRP appears to be only weakly related to liver function tests [40] and hepatic fat content, and is more strongly associated with visceral fat [68]. The association between CRP and incident diabetes may be stronger in women than men [4], but this needs further investigation. With respect to cardiovascular disease, there is ongoing debate about the added value of measuring CRP concentrations to improve risk prediction, with recent papers advising its use as premature [69, 70].

*IL-6* IL-6 is produced by a variety of cells, including adipocytes. Elevated IL-6 levels are associated with incident diabetes independent of obesity [3, 71–74] and fasting insulin [71]. In the BRHS, the relative risk of diabetes for individuals with IL-6 levels in the highest tertile (vs bottom tertile) was 2.02 (95% CI 1.14–3.58), following adjustment for BMI, lifestyle factors, pre-existing cardiovascular disease and systolic blood pressure [3]. Interestingly, further adjustment for HOMA-IR or CRP did not attenuate the relationship between IL-6 and diabetes.

If IL-6 is causally linked to diabetes, possible mechanisms include IL-6-mediated changes in insulin signalling in hepatocytes/adipocytes and central nervous system signals [75]. High IL-6 levels may also stimulate hepatic fatty acid synthesis and, as suggested by Yudkin and colleagues [76], cause endothelial dysfunction via vasocrine signalling. Chronically high IL-6 levels are linked to a range of metabolic abnormalities typical of an insulin-resistant state [77]. By contrast, the increase in IL-6 observed in myofibres during acute exercise appears to trigger anti-inflammatory and metabolically beneficial actions [78]. Thus, it is unclear whether IL-6 blockers would improve insulin sensitivity, but such studies would be helpful in assessing causal links.

*Other factors* Other acute phase response markers—raised white cell count, fibrinogen, orosomucoid and sialic acid, and low serum albumin—have also been linked to diabetes risk. Equally, measures of endogenous hormonal status are associated with diabetes. There is also interest in the association between diabetes risk and nutritional biomarkers such as plasma ascorbic acid (vitamin C) [79] and serum vitamin D [80]. However, plasma vitamin levels do not simply reflect intake and might be markers of other factors or lifestyle choices. Furthermore, studied associations may suffer from residual confounding from measured and unmeasured factors. One must therefore be cautious in inferring causal relationships. Notably, concentrations of many vitamins appear to be reduced when CRP levels are elevated, and so inflammation may be a confounder [81] (see text box). A comprehensive discussion of these factors is beyond the scope of this article.

*Some novel risk factors may be associated with impairments in normal insulin action* To further understand the links between novel parameters and an increased risk of type 2 diabetes, it is worth remembering that insulin interacts not just with liver, skeletal muscle and adipose tissue, but also with endothelium and immune cells. Thus, beyond glucose metabolism, insulin also (1) suppresses NEFA release from adipose tissue, (2) limits hepatic triacylglycerol synthesis, (3) helps maintain endothelial homeostasis, (4) regulates thrombotic cascades, and (5) may play a role in regulating inflammatory cascades. Alterations in several factors linked to incident diabetes could thus be at least partly explained by altered insulin function, such as elevations in triacylglycerol, PAI-1, CRP and adhesion molecules.

## Future research

Directions for future research are summarised in the text box ‘Key limitations of current research on novel biomarkers for diabetes, and suggested improvements’.

*Combining information from several biomarkers* Although most studies have tended to focus on single biomarkers for diabetes, benefit may be gained from combining information from several biomarkers. Such approaches could help identify genuine perturbances in relevant precursor pathways (e.g. when several liver-derived markers are raised in parallel), and as a result, predict diabetes risk with greater sensitivity and specificity. Of course, relevant studies require well-phenotyped biobanks containing accurate ascertainment of incident events.

*Potential for better insights* Notwithstanding the difficulties of collecting serial samples in large cohorts, dynamic changes in biomarkers may help to better determine which factors are more closely related to diabetes development in early vs later stages, and may help improve our understanding of the pathogenesis of diabetes. Of interest, Festa and colleagues [34] demonstrated that PAI-1 levels (but not fibrinogen) increased over 4 years in parallel with rising glucose levels and the development of diabetes. Similarly, we demonstrated that increases in ALT and triacylglycerol, over an 18 month period, accompanied progression to type 2 diabetes in men at risk, whereas weight, blood pressure, HDL-cholesterol, and white cell count, although higher at baseline, did not show significantly greater increases over time in men who developed diabetes [82]. Such findings further suggest that

hepatic fat accumulation is a contributing factor for conversion to diabetes in men at risk.

*Distinguishing between association and causality* It is important to reiterate that statistical association is not synonymous with causality. Future studies should address to what extent the novel factors fulfil criteria for causality, such as temporal association, dose response, reproducibility, independence, biological plausibility, specificity and reversibility. The 'Mendelian randomisation' approach [83], which combines information on genotype and related biochemical measures in prospective observational studies, has a role in addressing causality [84]. Of interest, genotypes predictive of higher CRP levels were not related to the metabolic syndrome or related factors in one study [85], although a separate study did report a significant association between a variant of the gene encoding CRP and incident diabetes [67]. As in the field of CHD, much larger numbers of incident diabetes cases and controls, accumulated from different populations, are needed to generate more robust findings.

*Diabetes intervention studies with pathway specific agents* The use of agents that specifically target one pathway or molecule, although uncommon, could help determine causality. For example, recent studies demonstrating an improvement in insulin resistance or related parameters (e.g. increase in SHBG) in response to TNF blockade in inflammatory conditions (such as rheumatoid or psoriatic arthritis) are promising [86–88]. The benefits of aspirin derivatives in diabetes are also being tested [89]. Interestingly, a recent controlled study on the use of a recombinant human interleukin-1-receptor antagonist in diabetes reported improvements in glycaemic status, beta cell secretory function and markers of systemic inflammation [90]. The emergence of other specific agents designed as therapies for inflammatory conditions (e.g. IL-6 blockade), could also be helpful in dissecting the relevance of low-grade inflammation in the pathogenesis of diabetes.

*Risk factor patterns for diabetes and vascular events differ: a weakness in the concept of the metabolic syndrome?* From the preceding discussions it should be apparent that many established predictors of diabetes, including measures of adiposity, fasting glucose and triacylglycerol, are more strongly linked to risk of incident diabetes than of vascular events. The same is also true for several novel risk factors, including adiponectin, adhesion molecules and, potentially, liver enzymes. As the former three routine measures are included in current metabolic syndrome criteria, this indicates that the metabolic syndrome is more strongly associated with risk of diabetes than of vascular events. This simple observation highlights the limitations associated with attempts to devise unified criteria (i.e. the metabolic syndrome) to identify individuals at risk of both CHD and diabetes. Further research comparing and contrasting risk factors for diabetes and vascular events is needed.

## Conclusion

We have reviewed the reported associations of many novel biochemical parameters with incident diabetes, and we suggest that many of these factors are interlinked; several provide an insight into ectopic fat accumulation, particularly hepatic fat, others into aberrant fat mass or function, and yet others into dysfunctional endothelial or immune function. Many parameters may also be influenced by aberrant insulin action in differing tissues. Given that few studies have simultaneously considered inter-related factors, the degree to which each is 'independently' associated with incident diabetes has, in general, been inadequately studied.

Moreover, little attention has been paid to measurement error or biological variation or potential differential associations of parameters dependent upon age, sex, adiposity or ethnicity. Furthermore, differential methods of diabetes diagnosis and degrees of adjustment for traditional risk factors limit the extent to which studies can be compared. The many limitations in published data suggest that systematic reviews would not be helpful at present. There is a need for more comprehensive and robust studies to address associations. Nevertheless, some of the findings have generated clinically useful information, for example better recognition that aberrant liver function tests—when seen in conjunction with obesity, modestly raised triacylglycerol or glucose—are commonly markers of excess hepatic fat. Equally, consistent links between elevated inflammatory parameters and diabetes onset have encouraged the development of novel therapies targeting immune pathways, some of which are beginning to show promise. Such targeted interventions and parallel genetic studies are needed to better attribute causality to novel biomarkers or pathways—epidemiological associations in isolation cannot achieve this.

By contrast, the use of novel biomarkers to help identify those at elevated risk of diabetes is very much in its infancy and cannot currently be recommended. Indeed, whether screening for prevalent diabetes, let alone for those at high future risk, will ever be widely adopted is by no means clear. If screening is implemented, it is likely to be performed for high-risk groups, such as overweight individuals with cardiovascular disease, and such individuals will already have several relevant measurements made. Therefore, for novel risk parameters to have any value, they must significantly improve risk prediction for future diabetes beyond simple risk factors that are easily available from

history or simple examination measures or blood tests. Alternatively, they must give additional insight into development of more rapid microvascular or macrovascular disease in patients with diabetes/non-diabetic hyperglycaemia, an area thus far poorly researched. Finally, even if novel risk factors do prove useful in risk prediction, issues such as cost–benefit relationships, and standardisation of tests, must first be considered.

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