Diet, obesity and endothelial dysfunction: of rats and men

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We read with interest in a previous issue of Clinical Science the paper by Naderali et al. [1] concerning the nutritional induction of obesity in Wistar rats and the subsequent relationship between hypertriacylglycerolaemia and endothelial dysfunction. Acknowledging the difficulties of drawing inferences for humans from studies carried out in experimental animals, the paper raises several interesting issues concerning the mechanisms whereby an unfavourable diet may lead to obesity and vascular disease. We should like to offer the following observations and viewpoint.

A principal correlate of endothelial dysfunction in the study appeared to be hypertriacylglycerolaemia. Hypertriacylglycerolaemia represents the accumulation of triacylglycerol-rich lipoproteins, some of which are atherogenic, e.g. chylomicron remnants, and others which are not, e.g. chylomicrons. The adverse effects of remnant lipoproteins on endothelial function and on clinical events have been shown previously in experimental [2] and human [3] studies respectively. Moreover, postprandial hypertriacylglycerolaemia may also be a consequence of the vascular insulin resistance of obesity [4], but whether this exclusively involves atherogenic lipoproteins remains unclear. Low high-density lipoprotein (HDL), a well-recognized reciprocal of hypertriacylglycerolaemia in obesity, has also been causally implicated in endothelial dysfunction [5]. It is likely that low HDL would have exacerbated the potentially adverse effect of hyper-remnantaeamia and other factors on the vasotonic responses noted in this study. One methodological drawback was that the authors did not specifically test in their ex vivo protocol the L-arginine/NO pathway by employing a specific inhibitor of NO synthase, such as \( \text{N}^\text{G} \)-monomethyl-L-arginine. The possibility of a specific defect in this pathway is also questioned on the basis of the abnormal vasotonic response to sodium nitroprusside, an endothelium-independent agonist. Vascular insulin resistance in vivo may also depend on an enhancement of endothelium-mediated vasoconstriction [6].

The induction of obesity in the study by Naderali et al. [1] did not appear to be related to insulin resistance, as measured using the homoeostasis model assessment (HOMA). This was a surprising finding, given that obesity was found to be associated with arterial vascular resistance. As recognized by the authors [1], insulin-mediated arterial vasodilation operates via the release of NO. In humans, endothelial dysfunction has accordingly been correlated with insulin resistance estimated by the hyperinsulinaemic euglycaemic clamp [7]. Fasting insulin or HOMA scores only offer an approximation of insulin resistance measured by the clamp, the so-called ‘gold standard’ test. In almost all studies of animal obesity, insulin-mediated glucose disposal, estimated by the hyperinsulinaemic euglycaemic clamp, has been shown to be impaired [8]. Another interesting finding was the lack of increase in plasma non-esterified fatty acids (NEFAs) with the induction of obesity. This is significant owing to the emerging role of NEFAs in inducing both insulin resistance and endothelial dysfunction, at least in humans [8,9]. The lack of increase in NEFAs may be related to decreased fatty acid cycling in the rat model, and is consistent with previous reports [8]. The study of Naderali et al. [1] was, however, at least 10-fold underpowered to demonstrate 30% differences in insulin resistance and plasma NEFA concentrations between obese and control rats, so that the likelihood of a false-negative finding is high. Nevertheless, there was clear evidence of an impairment in insulin-mediated vasorelaxation in the preconstricted mesenteric arteries of the cafeteria-fed rats. The metabolic correlates of this response were not reported, and it would have been interesting to know the potential roles of dyslipoproteinemia, adiposity and other variables.

Besides hypertriacylglycerolaemia, vascular insulin resistance and obesity, the study did not identify other features of the human ‘metabolic syndrome’, such as low HDL, hypertension and oxidative stress. Expression of a more comprehensive phenotype of this syndrome may require use of an alternative strain, such as the Koletsky

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rat. The potentially central role of oxidative stress is noteworthy. Visceral obesity is associated with increased cytosolic triacylglycerol storage in non-adipose tissue [10]. Accumulation of long-chain fatty acyl-CoA esters induces ADP deficiency by inhibiting mitochondrial adenosine translocation, and this increases mitochondrial oxygen free radical production. Increased oxidative stress may then result in both endothelial dysfunction and insulin resistance [10,11]. All abnormal vasotonic responses recorded ex vivo by Naderali et al. [1], including that due to nitroprusside, might have been consequent on increased endothelial and/or smooth muscle cell production of superoxide radicals. We have argued elsewhere [12] that this mechanism may also be causally involved in the pathogenesis of diabetic endotheliopathy.

Another limitation of the study by Naderali et al. [1] was that the assessment of vascular function was carried out ex vivo. This would not have allowed the contribution of systemic endocrine effects that occur in obesity to be studied, in particular the effects of increased sympatho-adrenal activity that can impair microcirculatory function [13]. Against this, the authors [1] did carry out their experiments in arteries preconstricted with noradrenaline, although this would not have mimicked quantitatively the systemic effects of catecholamines in human obesity. In relation to the use of mesenteric arteries, one must query whether such vascular responses reflect responses in other arterial beds, particularly the coronary circulation. Human data suggest, however, that vasotonic dysfunction in peripheral arteries may be a surrogate for coronary arteries [14].

Aside from the central hypothesis tested in their study [1], an interesting finding was the divergence in total body weight in the rats fed an equivalent cafeteria diet. Given that total body weight gain was apparently bimodally distributed, but gonadal and perirenal fat masses were similar in the two cafeteria-fed groups, the possibility arises of differences in the accumulation of subcutaneous fat. Weight gain in women, who tend to accumulate fat subcutaneously, has recently been demonstrated to depend on mutations in the $\beta_3$-adrenergic receptor and uncoupling protein-1 genes [15]. Although gonadal and perirenal fat mass were equally predictive of abnormal vasotonic responses to acetylcholine in the study of Naderali et al. [1], infra-abdominal fat was not recorded, probably owing to technical difficulties, and its contribution to endothelial dysfunction might have been greater than other fat compartments. It would also have been useful to know the precise nutrient composition of the experimental diet, since both saturated and mono-unsaturated fats have been linked to the development of obesity [16], and in the postprandial state they independently impair endothelial function in humans [17].

In summary, it is likely that the vascular abnormalities reported in this study [1] are due to insulin resistance and its atherogenic consequences, i.e. hyper-remnantaemia, low HDL and hyperoxidative stress. A larger sample size and use of the hyperinsulinemic euglycaemic clamp together with other measurements suggested above would strictly be needed to support our contention. A gender difference in the topography of obesity is well recognized in humans, and it is possible that stronger relationships between endothelial dysfunction, hypertriacylglycerolaemia and visceral adiposity might have been found had male rats been investigated. Finally, while this study concerned the induction of obesity and its vascular and metabolic sequelae, it is interesting to speculate whether in another experimental design these abnormalities could be reversed with weight loss. Preliminary data in human subjects suggests that this may be the case [18].

REFERENCES


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Many animal and human studies have shown that obesity has deleterious effects on vascular function and structure [2–4]. Vascular abnormalities vary widely in different studies [4,5], which may point to the importance of the diet content, strain of the animals used and the duration of the experiments [1,4]. We and others [5–8], have shown that diet-induced obesity impairs endothelium-dependent, agonist-induced vasorelaxation, while having no effects on vascular contractility. Evidence from our group [6–8] and others [9–11], suggests that the obesity-related defect in vasorelaxation at the endothelium level is primarily NO-dependent [12,13], and that vascular dysfunction also affects smooth muscle cells [8,12,14,15].

Various animal studies [4,8,16] have shown that the severity of diet-induced insulin resistance depends on the type of diet and the duration of the study. Our present study [1] aimed to determine whether or not increased adiposity is the critical factor in ‘obesity-related’ vascular dysfunction. Our data [1] clearly indicate that consumption of a highly palatable diet can markedly impair vascular function before it results in any increase in fat mass. This clearly points the finger at specific dietary components or the body’s response to these. The insulin resistance that is regarded as central to the metabolic syndrome is one candidate [17]. However, we have questioned its importance in diet-induced vascular dysfunction [18], as typical endothelium-dependent abnormalities were found in rats fed a palatable diet, in the absence of insulin resistance, measured both by the homoeostasis model assessment (HOMA) index, as well as by the hyperinsulinaemic euglycaemic clamp [18]. This finding is in contrast with the report published by Oakes et al. [19]. One explanation for these contrasting results may relate to the content of the diet used in the respective studies. Oakes et al. [19] chronically maintained their rats on a high-fat diet, whereas ours [1] was a highly palatable diet, but low in fat content (16% versus 59%).

As Watts and Redgrave point out, the lack of increase in plasma non-esterified fatty acids (NEFAs) in our study [1] was somewhat unexpected. We have previously shown that plasma levels of both triacylglycerols and NEFA increase in diet-induced obesity [7,8,18], and that increases in the former are particularly marked. These findings are consistent with many other data indicating the importance of hypertriacylglycerolaemia in obesity- and diabetes-related vascular dysfunction [1,18,20–24]. Nonetheless, we are not excluding an association between

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rises in NEFA and vascular dysfunction in dietary-induced obesity [18].

We also recognize a possible role for oxidative stress in endothelial dysfunction. Various reports have indicated that antioxidants can preserve vascular function [25–27], whereas others have shown that reducing oxidative stress fails to improve endothelial function in Type I diabetes mellitus [28,29]. However the precise mechanism(s) of action of the deleterious effects of oxidative stress on vascular function still remains largely unknown, and our study [1] was not designed to investigate this possibility.

Like many researchers, we used a well-established experimental protocol which is widely employed in in vitro studies of vascular function [1,5,6,9,10,28,30], in which relaxation is measured in arteries preconstricted with noradrenaline. The use of the mesenteric artery is justified by the contribution of this class of arteries to peripheral resistance and thus to cardiovascular disorders [30].

In summary, we contend that the vascular abnormalities seen in our study [1] are not due to insulin resistance itself. This conclusion is backed up by the unchanged insulin sensitivity (measured by HOMA and the clamp) in our most recent report [18]. That study was larger and used both male and female rats, which should help to allay Watts’ and Redgrave’s concerns about sample size and gender. Finally, we agree with Watts and Redgrave that further studies are needed to investigate whether diet-induced vascular abnormalities are reversible. Such a study has recently been completed in our laboratory and we hope that its results will help to clarify their interesting suggestions.

REFERENCES


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