

## Why serum chemokine levels are raised in insulin resistance syndrome: An immune reversal hypothesis

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*The adipose tissue is an active endocrine organ which also secretes proinflammatory cytokines and chemokines resulting into raised serum levels. Although the pathological role of adipocyte-mediated immune changes in the insulin resistance syndrome or metabolic syndrome is being increasingly recognized, its functional significance in healthy life remains yet unexplained. We suggest a functional role for adipocyte secretion of chemokines based on the behavioural switch hypothesis for the evolutionary origin of insulin resistance. According to this hypothesis, insulin resistance is a physiological adaptation to 'soldier to diplomat' transition in lifestyle. The 'soldier' life is more prone to wounds and injuries. Therefore the immune system should be deployed more in the sub-cutaneous tissue. In 'diplomat' life, where cutaneous injuries are less likely, the immune system could be retracted from the periphery. We suggest here that chemokine secretion by adipocytes is one of the mechanisms of this immune reversal. In a physically active lifestyle, stimulated by minor cutaneous injuries, immune cells move towards the periphery under a chemokine gradient formed by the chemokine secretion by the injured tissue. A gradient results from the difference between local and basal levels of chemokines. Secretion of these chemokines by adipocytes increases the basal level, thereby weakening the gradient. Using diffusion kinetics we show that a small rise in basal levels can cause substantial reduction in cell infiltration. This response may have evolved as a mechanism of disinvestment in peripheral immunity resulting into an immunological parsimony.*

Recent research has compelled some radical rethinking about the evolution and basic biology of the insulin resistance syndrome or the metabolic syndrome<sup>1</sup>. Many of the comorbidities of the metabolic syndrome are now known to be due to inflammatory changes<sup>2-5</sup>. There is said to be a low-grade systemic inflammation and serum levels of various inflammatory cytokines, chemokines and inflammatory markers, including TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, IL-10, MCP-1, MIP-1 $\alpha$ , GRO- $\alpha$ , IP-10 and CRP are increased. Adipocytes are active secretors of various inflammatory and chemotactic cytokines and a substantial portion, if not all, of the raised serum levels is contributed by the adipose tissue<sup>6-10</sup>.

So far no satisfactory explanation is available as to why adipose tissues secrete inflammatory chemokines. If this property has been consistently shown, it must have evolved under some kind of selective pressure. Chemokine secretion by adipocytes must be adaptive under certain sets of conditions, although it is pathological in the context of metabolic syndrome.

It has been argued repeatedly that insulin resistance evolved as an adaptive response under some sets of conditions and with the modern lifestyle it is turning detrimental<sup>1,11</sup>. The dominant paradigm for over 40 years has been that it evolved

as a thrifty tendency to cope with the alternating 'feast and famine' situation and in the modern 'feast and feast' scenario, it turned pathological. The thriftiness family of hypotheses was recently challenged by Watve and Yajnik<sup>1</sup> on several grounds. However, they agree with the basic tenet that insulin resistance evolved as an adaptation and turned pathological owing to a changed lifestyle. Watve and Yajnik<sup>1</sup> further proposed a behavioural switch hypothesis which suggests that insulin resistance evolved as an adaptation for a 'soldier to diplomat' transition in behaviour, i.e. a shift from muscle-dependent to brain-dependent lifestyle.

There have been attempts to explain the hypercytokinemia under the thriftiness paradigm. It has been interpreted as raised level of innate immunity<sup>12</sup> and its evolution is interpreted as a response to increased chances of infection under starvation conditions in which the thrifty phenotype evolved<sup>13</sup>. If starvation and infection challenges co-occurred during the hunter-gatherer life, thrifty genotype and infection-resistant genotype may have co-evolved. An inherent weakness of this explanation is that in obesity or insulin resistance there is no evidence of increased resistance to infections. The raised levels of inflammatory cytokines have not been demonstrated to confer increased resistance to infections. On the

contrary, many specific infections including those of the skin are more common in diabetic patients<sup>14</sup>. Other infections occur with increased severity and are associated with an increased risk of complications in patients with obesity and diabetes<sup>15</sup>. Therefore, there appears to be an immune imbalance<sup>16,17</sup> rather than enhanced immunity.

The behavioural switch hypothesis attempts to explain the inflammatory changes in a different way. In a 'soldier' life, the risk of getting wounds and injuries is substantially greater compared to a 'diplomat' life. Therefore, the immune cells should move to the periphery, i.e. towards the subcutaneous tissue. Whenever there is a shift to a diplomat life, the immune system should retract from the periphery and as a result would be centrally more active. This, according to Watve and Yajnik<sup>1</sup>, results into delayed wound healing on the one hand, and increased inflammatory tendency of central organs on the other.

Can we test the hypothesis? First of all, does a negative association or a trade-off between cutaneous and central immunity exist? If yes, does it change in obesity and metabolic syndrome? If yes, what are the molecular mechanisms involved?

There is some circumstantial evidence for trade-off or shift of balance among the innate immune mechanisms in obe-

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sity and insulin resistance (Figure 1). Effective wound healing needs a balance between the mechanism of inflammation and that of wound healing and closure. An enhanced inflammatory reaction impairs wound closure, and premature wound closure before getting rid of invading microorganisms may lead to sepsis. In obesity-related disorders there appears to be a shift in this balance against wound healing<sup>18</sup> and towards inflammation<sup>2-10</sup>. A number of other observations are also suggestive of a shift of immune balance. There is a negative association between atopic allergy and obesity<sup>19</sup>. Delayed-type hypersensitivity is also reduced in both type-1 and type-2 diabetes<sup>20,21</sup>, suggesting that at least some of the components of the immune system are partially retracted from the skin.

One of the major players in the immune redistribution is likely to be testosterone. Braude *et al.*<sup>22</sup> suggested that testosterone may be an immune redistributor rather than an immunosuppressor. According to their hypothesis, testosterone brings about movement of the immunological garrison to the periphery, since testosterone is associated with male aggression and therefore predicts wounds. Although there have been hardly any attempts to seriously test the hypothesis of Braude *et al.*<sup>22</sup>, there is some circumstantial evidence in its favour. On the one hand, serum testosterone levels are negatively associated with serum inflammatory markers and other components of the metabolic syndrome<sup>23,24</sup>. Testosterone is shown to be protective against inflammatory complications of the metabolic syndrome<sup>24,25</sup>. On the other hand, although testosterone is generally considered immunosuppressive, it is shown to enhance inflammation (but not wound healing) of cutaneous wounds<sup>26-28</sup>. Thus the action of testosterone on inflammation appears to be in opposite directions in the context of skin and that of central organs.

The peroxisome proliferator activator receptors (PPARs) appear to be another major class of players in a shift of balance. PPARs arrest inflammation on the one hand<sup>29</sup>, and enhance wound healing and wound closure on the other<sup>30,31</sup>. Epidermal injuries activate the expression of PPARs and especially that of PPAR- $\beta$ <sup>31</sup>. Absence of injuries on adopting a diplomat way of life may shift the immune balance against healing and towards inflammation by arresting PPAR expression (Figure 1).

Sensory nerves and neuropeptides also play a significant role in modulating inflammation and wound healing<sup>32-35</sup>. In diabetes, there is a progressive degeneration of peripheral nerves<sup>36</sup>, shifting the immune balance further away from the skin.

The possible trade-off of immunity between skin and central circulation suggests a possible role for chemokine secretion by the adipose tissue. Movement of immune cells is under a gradient of chemokines<sup>37-40</sup> secreted at the site of injury by resident tissue cells. They are locally retained on matrix and cell surface heparan sulphate proteoglycans, establishing a concentration gradient around the stimulus. Chemokine signalling activates leukocyte integrins, leading to extravasation. The magnitude of the gradient is determined by the difference between the local level and the baseline blood level. We hypothesize that if the basal levels of chemokines are high, the gradient will be weak and migration will be downregulated. Adipocyte secretion of chemokines increases the basal level and thereby arrests the flow of inflammatory cells towards the periphery. Fat accumulation is a sign of a lifestyle and behavioural change and therefore, it is the right kind of tissue to bring about the redistribution of the immune system. Diabetes is known to reduce late-phase inflammation of

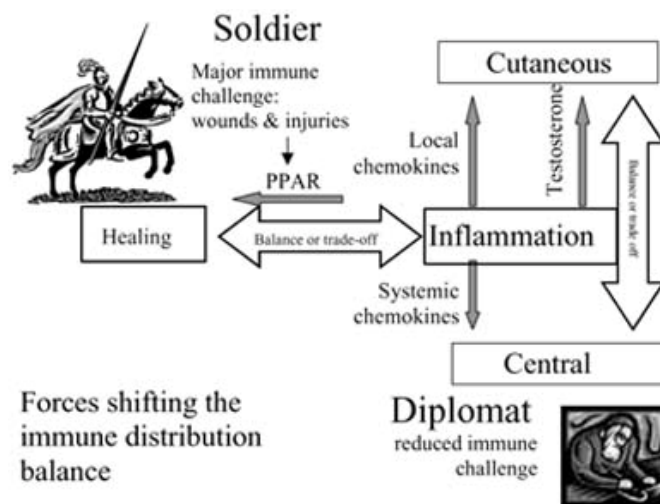
wounds<sup>41</sup> and this may be due to the raised basal levels of chemokines.

The action of chemoattractants and other signalling molecules largely depends upon their environments, and relative rather than absolute concentrations. Increased expression of a signalling molecule need not always mean increased action. The local topology of its relative concentrations can be of utmost importance. The effects of local application of TNF- $\alpha$  are substantially different from those of systemic administration. In a mice study, local application of 50–500 ng of TNF- $\alpha$  in collagen improved wound healing, but similar quantity in phosphate buffered saline or 75  $\mu\text{g}/\text{kg}$  intraperitoneally did not<sup>42</sup>. Presumably, TNF- $\alpha$  in collagen was able to form a stable gradient, whereas that in saline dispersed systemically too rapidly and systemic TNF- $\alpha$  had no detectable effect.

We will now analyse using diffusion kinetics, the effects of raised basal chemokine levels on the gradient formation and chemotactic migration of cells. If we assume simple diffusion of a chemoattractant, gradient formation can be computed using Fick's law of diffusion<sup>43</sup>.

$$X^2 = 4Dt \ln(C_o/C_x),$$

where  $X$  is the distance from the origin of the chemoattractant,  $D$  the diffusion con-

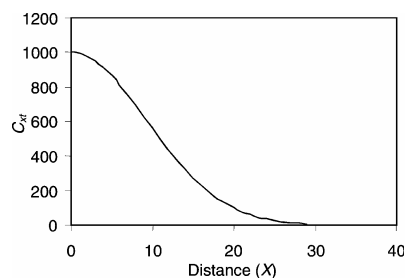


**Figure 1.** Schematic representation of the expected shift in immune balance on adopting a phenotypic transition from 'soldier' to 'diplomat' life: In 'soldier' life the major immunological challenges are peripheral wounds and injuries for which the immune system should be deployed peripherally. In 'diplomat' life where peripheral injuries are much unlikely, peripheral immunity would be an unnecessary investment and should be retracted. Therefore, a shift in balance is expected from wound healing to inflammation and from periphery towards central organs. Possible mediators of this change, namely testosterone, PPARs and chemokines are indicated.

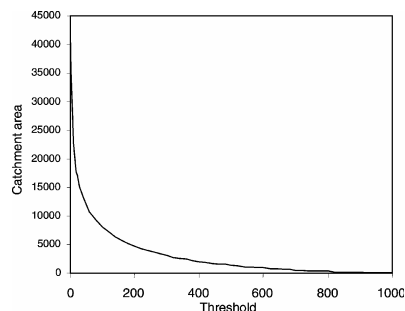
stant,  $t$  the time of diffusion,  $C_o$  the concentration at the origin and  $C_{xt}$  the concentration at distance  $X$  in time  $t$ .

It can be seen that the concentration gradient thus formed is nonlinear and highly concave towards the lower end (Figure 2). We assume that there is a threshold level of concentration difference which is necessary for the cells to recognize a gradient. A sphere (or hemisphere) of radius equal to the distance between the source and the threshold forms the 'catchment volume' of cell recruitment. The distance between the origin and threshold concentration will be the radius of this hemisphere. Therefore, the catchment volume will rise in cubic proportion of  $X$ . A larger catchment volume will result in greater infiltration of cells.

The threshold difference must depend upon the basal levels of the chemoattractant. If the basal levels rise, the threshold for chemotaxis should also increase. The concave nature of the concentration gradient results into a nonlinear reduction in the catchment volume, such that when the threshold is small even a little incre-



**Figure 2.** Decreasing concentration of a chemoattractant from the point of origin assuming Fick's law of diffusion (both distance and concentration in arbitrary units).



**Figure 3.** Decrease in the catchment area with increasing threshold assuming diffusion kinetics as in Figure 2. The source concentration is assumed to be a thousand fold compared to the minimum possible threshold.

ment in it can cause a large decrement in the catchment. But when the threshold is high, a large change is required to cause the same decrement in the catchment (Figure 3).

Data on normal and obesity-induced levels of TNF- $\alpha$ , IL-6 and MCP1 show that there can be a two- to tenfold rise in their basal levels in obesity<sup>10,44</sup>. For example, Bastard *et al.*<sup>44</sup> found that the mean levels of TNF- $\alpha$  in obese patients ( $1.48 \pm 0.15$  pg/ml) were about double the mean for lean controls ( $0.74 \pm 0.09$  pg/ml). Those for IL-6 were  $0.39 \pm 0.06$  pg/ml for lean controls and  $3.58 \pm 0.51$  pg/ml for obese diabetic patients. Data on their concentrations at the point of origin ( $C_o$  of the above model) are difficult to obtain, but there are indications that the difference could be of many orders of magnitude compared to the healthy basal levels<sup>42</sup>. Assuming a thousand-fold difference between the source and threshold concentrations, we take the healthy basal level as 1 arbitrary unit and the source concentration as 1000 units. We further assume that the threshold difference is directly proportional to the basal level, with a proportionality constant of unity. With these assumptions, if the threshold levels are raised to 2 or 10 units, the model computes that there would be about 15 and 45% reduction in cell infiltration respectively. Therefore, a small cost of raising the blood levels of chemokines can save a much greater investment in peripheral immunity. Raising the basal levels of chemokines can result in immunological parsimony.

Two other changes associated with obesity and type-2 diabetes also seem to work in the same direction. There is a reduction in chemokine production by tissue macrophages<sup>45-47</sup>. If there is reduction in local chemokine secretion at the site of injury, it would weaken the gradient from the distal end also. There is also an increase in the level of soluble receptors of some of the cytokines<sup>48</sup>, which is expected to interfere further in signaling. All these changes together appear to make a concerted effort to reduce migration of cells involved in innate immunity.

Thus the raised levels of chemokines and pro-inflammatory cytokines may actually reduce the effect of chemokine action, rather than enhancing it. TNF- $\alpha$  is often taken as an inducer of insulin resistance. If this were the case, impairment of TNF signalling should have a protective effect. However, TNF- $\alpha$  receptor

knockout mice have been shown to be more susceptible to obesity-induced diabetes<sup>49</sup>, indicating that TNF- $\alpha$  action may actually protect against diabetes. The increased basal levels of TNF- $\alpha$  during obesity may reduce its protective effect rather than enhancing it.

Compatible with the hypothesis are the observations that the total monocyte count in blood goes up in a number of obesity and diabetic complications<sup>50,51</sup>, whereas macrophage density in peripheral tissues decreases<sup>47</sup>. Total leukocyte count as well as neutrophil count in blood is also positively associated with diabetic micro- and macrovascular complications<sup>52,53</sup>. Reduced migration of both monocytes and neutrophils is likely to be responsible for this. Leukocyte chemotaxis is demonstrably down-regulated in both type-1 and type-2 diabetes<sup>54,55</sup>. A number of mechanisms are likely to be involved in the altered cell migration, raised basal levels of chemoattractants being one of them.

A sedentary lifestyle devoid of physical activity and physical aggression creates a condition in which peripheral injuries are less likely. Obesity is prevalent under similar lifestyle. Therefore, the appropriate tissue to signal an immune reversal response to a non-aggressive sedentary lifestyle would be the adipose tissue. However, this change may have associated hazards. The basal levels of chemokines cannot be expected to be stable. They will be subject to stochastic spatiotemporal fluctuations. The fluctuations are bound to increase in amplitude with rising basal levels. A stochastic peak exceeding the threshold can result into a non-specific inflammatory trigger. This is a chance event that can occur anywhere in the body at any time. Therefore, raised basal levels can result in stochastic inflammatory responses that can turn pathological. The modern urban lifestyle is much more sedentary and much less injury-prone compared to the conditions in which we evolved. It is possible therefore, that this lifestyle acts as a supernormal stimulus for immune reversal. An exaggerated immune reversal can lead to pathological consequences.

There are three possible lines of work by which the hypothesis can be tested. First, in animal models, it should be possible to test experimentally whether raising basal blood levels of chemokines by infusion decreases infiltration of immune cells to an experimental wound. This can

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be the most direct test of the hypothesis. As a long-term consequence of the altered basal levels, the distribution of immune cells in the body, mainly macrophages, is likely to change in obesity and related disorders. By appropriate labelling of macrophages followed by a whole-body scan, one could test in animal models whether the distribution of macrophages and other immune cells is different in healthy versus obese insulin-resistant individuals. Also, on an epidemiological scale, it can be tested whether continued long-term exposure to minor cutaneous injuries is protective against obesity-induced insulin resistance and the accompanying inflammatory complications. There is already some evidence that minor cutaneous injuries and skin stimuli resulting from diverse causes such as insect bites, bee-stings or acupuncture have systemic anti-inflammatory effects<sup>56,57</sup>. If the hypothesis stands rigorous testing, it can potentially bring about a paradigm shift in the basic biology as well as prevention, control and treatment of obesity-related disorders. Arresting immune reversal and redirecting the innate immune system to the periphery using sensory, behavioural and biochemical stimuli may turn out to be a useful strategy in the treatment, and possibly reversal of insulin resistance syndrome.

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