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Mining microarray datasets in nutrition: expression of the *GPR120* (*n*-3 fatty acid receptor/sensor) gene is down-regulated in human adipocytes by macrophage secretions

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Abstract

Microarray datasets are a rich source of information in nutritional investigation. Targeted mining of microarray data following initial, non-biased bioinformatic analysis can provide key insight into specific genes and metabolic processes of interest. Microarrays from human adipocytes were examined to explore the effects of macrophage secretions on the expression of the G-protein-coupled receptor (GPR) genes that encode fatty acid receptors/sensors. Exposure of the adipocytes to macrophage-conditioned medium for 4 or 24 h had no effect on *GPR40* and *GPR43* expression, but there was a marked stimulation of *GPR84* expression (receptor for medium-chain fatty acids), the mRNA level increasing 13·5-fold at 24 h relative to unconditioned medium. Importantly, expression of *GPR120*, which encodes an *n*-3 PUFA receptor/sensor, was strongly inhibited by the conditioned medium (15-fold decrease in mRNA at 24 h). Macrophage secretions have major effects on the expression of fatty acid receptor/sensor genes in human adipocytes, which may lead to an augmentation of the inflammatory response in adipose tissue in obesity.

Key words: **Adipocytes: Fatty acid receptors: Inflammation: Microarrays**

DNA microarrays, which have been increasingly used to examine global gene expression in different areas of nutritional science, are a rich source of information. Bioinformatic analysis of microarray datasets provides an insight into the major networks and key pathways that are altered in a particular situation or in response to a specific stimulus – whether nutritional, hormonal or environmental. These pathways and networks, together with the identification of the genes whose expression is altered most substantially, are usually of most interest. This is undoubtedly the case during the first phases of analysis, and the ensuing publication(s) will invariably focus on the major changes occurring in response to the particular stimulus. Frequently, there may be little further mining of the datasets. However, continued exploration of microarray data can yield considerable additional information and provide unexpected insight, either on specific genes and groups of genes, or on less prominent pathways and networks. This does not necessarily require complex bioinformatics, but may relate to a targeted interest in particular genes or metabolic processes.

An example of this additional insight comes from studies that we have conducted on human adipocytes exposed to an inflammatory stimulus in the form of incubation with macrophage-conditioned medium[(1)]. The initial bioinformatic analysis indicated that the pathways that were most strongly modulated on exposure of the fat cells to signals from

Abbreviation: GPR, G-protein-coupled receptor.

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macrophages are those associated with inflammation, glucose uptake and macrophage infiltration[1]. The most highly up-regulated genes were a group of matrix metalloproteinases: the *MMP1*, *MMP3* and *MMP10* genes each exhibited >1000-fold increase in mRNA level in the adipocytes after 24 h exposure to macrophage-conditioned medium, while 200–400-fold increases in *MMP9* and *MMP12* expression were evident[1]. Subsequent analysis of the datasets has indicated that the expression of genes associated with vitamin D_3 metabolism is also modulated in the adipocytes by macrophage secretions. There was, in particular, a substantial stimulation in the expression of the *CYP27B1* gene (82-fold increase in mRNA level after 24 h) which encodes the 25(OH)D_3-1α-hydroxylase that catalyses the conversion of 25-hydroxycholecalciferol to the active vitamin D_3 hormone, 1,25-dihydroxycholecalciferol[2]. Expression of the nuclear vitamin D receptor gene, *VDR*, was also increased (7·7-fold elevation in mRNA after 24 h). These observations indicate that the ability of adipocytes to synthesise the biologically active vitamin D_3 hormone, and to act as a target for the hormone, is markedly stimulated during inflammation, consistent with an anti-inflammatory action of vitamin D_3 in adipose tissue[2].

Further targeted mining of the microarrays for genes associated with nutrient metabolism has revealed insight into the effects of macrophage secretions on the expression in adipocytes of the G-protein-coupled receptors (GPR) that act as fatty acid receptors and sensors. These GPR fatty acid receptors/sensors have been the focus of considerable interest recently, particularly in relation to inflammation and insulin sensitivity, and several are now recognised – GPR40, GPR41, GPR43, GPR84 and GPR120, each of which has a different selectivity[3]. GPR40 (also known as FFAR1) binds long-chain fatty acids[4,5], while GPR41 (FFAR3) and GPR43 (FFAR2) are receptors for SCFA[6,7]. GPR84 is selective for medium-chain fatty acids[8], while GPR120 has recently been shown to be a receptor for *n*-3 PUFA[9]. Each of the GPR fatty acid receptors has been reported to be expressed in (white) adipose tissue, with strong expression, relative to other tissues, of GPR43 and GPR120 in particular[4,6,8,9]. GPR120 is expressed strongly in white adipocytes (3T3-L1 cells) themselves, expression being differentiation dependent; it is also highly expressed in macrophage and monocyte cell lines[9]. Although a high expression of GPR41 was originally reported in adipose tissue, subsequent studies have found little or no expression in the tissue[9–11].

Several roles have been demonstrated for the different GPR fatty acid receptors/sensors. For example, GPR40 is involved in the glucose-induced stimulation of insulin secretion from pancreatic β-cells[5], while SCFA stimulate glucagon-like protein-1 secretion from colonic cells and leptin secretion from adipocytes through GPR43[12]. Both GPR84 and GPR120 have been implicated in inflammation. A recent report has indicated that in murine 3T3-L1 adipocytes incubation with TNFα, or co-culture with a macrophage cell line, leads to marked stimulation of *GPR84* gene expression, with medium-chain fatty acids inhibiting adiponectin expression via this receptor[13]. More importantly, GPR120 has been shown to mediate the anti-inflammatory and insulin-sensitising effects of *n*-3 PUFA, ameliorating inflammation-induced insulin resistance in obesity[9]. GPR120 knockout mice are more insulin resistant than wild-type controls, and when fed a high-fat diet supplementation with *n*-3 fish oils did not reverse their insulin resistance, in contrast to the wild-type animals[3,9]. The knockout mice are also reported to develop obesity on a high-fat diet, and exhibit a fatty liver and reduced adipogenesis[14].

Analysis of our microarray datasets showed no change in GPR40 and GPR43 mRNA levels following exposure of human adipocytes to macrophage-conditioned medium (Table 1); no signal was obtained for GPR41. However, increases in *GPR84* expression were observed, at both 4 and 24 h of incubation with conditioned medium, the mRNA level being increased 13·5-fold at 24 h relative to the adipocytes incubated with unconditioned medium (Table 1). This observation, from human adipocytes, is consistent with the demonstration that *GPR84* gene expression in 3T3-L1 adipocytes is stimulated by TNFα and macrophage-secreted factors[13]. Up-regulation of GPR84 receptor expression by inflammatory mediators would be predicted to lead to an accentuation of the inflammatory response in fat cells.

In contrast to the other GPR fatty acid receptors, there was a marked reduction in *GPR120* expression in human adipocytes exposed to macrophage-conditioned medium, the mRNA level being reduced by as much as 15-fold after 24 h (Table 1). Thus, macrophage-secreted factors induce a substantial down-regulation of the expression of the gene encoding the major receptor/sensor for *n*-3 fatty acids. The decrease in the receptor (assuming that the changes in gene expression are accompanied by a reduction in the receptor protein) in adipocytes would presumably, and paradoxically, compromise the well-recognised and extensive anti-inflammatory action of these fatty acids[15,16]. This suggests that such fatty acids may have a more limited inhibitory effect on inflammation and insulin resistance in adipocytes, at least that induced by macrophages, than might be expected. The microarray data based on the response to macrophage-conditioned medium needs to be followed by a detailed investigation of the effects of more specific inflammatory stimuli, such as

Table 1. Effect of macrophage-conditioned medium on the expression of G-protein coupled fatty acid receptor genes in human adipocytes†

Gene	Fold-increase in mRNA at 4 h	Fold increase in mRNA at 24 h
GPR40	1.2	1.1
GPR41	nd	nd
GPR43	1.0	1.1
GPR84	5.5**	13.5**
GPR120	0.8	0.07**

nd, Not detected.
† Gene expression was determined with Agilent microarrays following incubation of human Simpson–Golabi–Behmel syndrome (SGBS)[19] adipocytes with macrophage-conditioned medium, or unconditioned medium (controls), for either 4 or 24 h[1]. The adipocytes were differentiated from fibroblastic preadipocytes in primary culture and were used at 10 d after the induction of differentiation. The fold-change relates to the conditioned medium compared with unconditioned medium (controls).
** Value was significantly different from that of controls ($P < 0.01$).

lipopolysaccharide and TNFα, on adipocyte *GPR120* expression and cellular GPR120 protein content. This includes the interaction with *n*-3 fatty acids.

It is noteworthy that macrophage secretions simultaneously up-regulate *GPR84* and down-regulate *GPR120* expression in human adipocytes, both effects being likely to lead to an amplification of the fat cell inflammatory response. The present analysis highlights the involvement of the GPR fatty acid receptors/sensors in inflammation in adipose tissue, and demonstrates clear differences in response in adipocytes between the key receptors/sensors. It also raises the possibility that the anti-inflammatory effect of *n*-3 fatty acids from fish oils may be attenuated in adipose tissue in obesity because of the extensive recruitment of macrophages into the tissue[17,18]. Finally, the present article illustrates the richness of information that is available in microarray datasets and which can be obtained in nutritional (and other) studies by repeated, targeted mining without the need for complex bioinformatic analysis.

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