Diet-induced Obesity Up-regulates the Abundance of GPR43 and GPR120 in a Tissue Specific Manner

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Key Words

GPR43 • GPR120 • Skeletal muscle • Heart • Liver • Type 2 diabetes mellitus • Obesity • mRNA

Abstract

Background/Aims: GPR43 and GPR120 have recently been orphaned as receptors for fatty acids. Fatty acids mediate a variety of metabolic processes in the body, however, the effect these receptors have on metabolism is not fully understood. Here, we characterise the effect of diet-induced obesity on the expression of GPR43 and GPR120 in tissues important in maintaining metabolic health. **Methods:** Six-week old male Sprague Dawley rats were fed either a high fat diet (HFD; 22% fat) or control diet (5% fat; n = 8-9/group) for 12 weeks. Rats were euthanized and the heart, liver, soleus and extensor digitorum longus (EDL) skeletal muscles were excised. GPR43 and GPR120 receptor abundance was quantified by 'real-time' PCR. **Results:** GPR43 mRNA abundance was significantly up-regulated by a HFD in liver and soleus and EDL skeletal muscles compared to control ($p \leq 0.05$). Whilst a HFD significantly up-regulated GPR120 gene transcripts in cardiac tissue and EDL skeletal

muscle when compared to control ($p \leq 0.05$). **Conclusion:** We have shown for the first time that up-regulation of GPR43 and GPR120 in response to a HFD, is tissue specific. This suggests these receptors have different roles in mediating metabolic function in a number of tissues in the human body.

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Introduction

Obesogenic changes in diet and lifestyle have seen the global prevalence of obesity, type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) reach epidemic levels in recent decades (reviewed by [1-3]). It is recognised that the obese and diabetic disease states elicit significant detrimental effects on systemic metabolic homeostasis which appreciably increases risk of morbidity and premature mortality [4, 5]. In lieu of this, it has become highly pertinent to investigate new endogenous physiological targets aimed at the safe treatment of

obesity, T2DM and associated co-morbidities such as CVD.

G-protein coupled receptors (GPCR) constitute a physiologically diverse family of receptors within the body [6]. Endogenously, this class of receptors are activated via a large range of ligands including ions, bioamines, odorant molecules, nucleotides, peptides/proteins, carbohydrates and fatty acids (reviewed by [6-8]). This high degree of heterogeneity enables GPCRs to mediate metabolic homeostasis through a large array of mechanisms [9]. GPCRs are highly amenable to selective manipulation by synthetic compounds and are the target of approximately half of all recently developed drugs [10]. As such GPCRs are at the forefront of research aimed at the treatment of metabolic diseases including obesity, T2DM and CVD.

GPR43 (also designated free fatty acid receptor 2) and GPR120 are recently orphanised GPCRs which have been identified as receptors for endogenous free fatty acids (FFA) [11-17]. FFAs are generated during lipolysis [18] and enter the bloodstream to circulate throughout the body. Circulating FFAs not only provide substrate for energy production but importantly can also act as lipid sensors and mediate the expression of genes and proteins to regulate lipid and energy homeostasis in a diverse range of physiological and pathophysiological conditions [19, 20]. Recent research has shown that GPR43 and GPR120 are activated by structurally discrete fatty acids with GPR43 being activated by short chain fatty acids (SCFAs), with acetate exerting the greatest specificity for GPR43 [13, 14]. Conversely, long chain fatty acids (LCFA) have been identified as the endogenous ligands for GPR120 including the omega 3 fatty acids alpha-linolenic acid (α -LA) and docosahexaenoic acid (DHA) [15-17]. These findings have resulted in GPR43 and GPR120 receiving significant interest for their therapeutic potential in the treatment of obesity, T2DM and their associated co-morbidities.

GPR43 and GPR120 exhibit a unique tissue distribution which is reflected in their known functions. The GPR43 receptor is abundantly expressed in immune tissues [21-23], enteroendocrine cells of the gastrointestinal tract [24] and adipose tissue [23, 25]. GPR120 tissue expression shares some similarities with that of GPR43, with GPR120 mRNA being detected in high amounts in adipose tissue [16, 26], tissues of the gastrointestinal tract, lung [17, 26] and the pituitary [26].

Accordingly, both GPR43 and GPR120 have been demonstrated to mediate adipocyte differentiation with their activation eliciting an adipogenic effect [23, 26, 27].

In addition these receptors modulate secretion of gastrointestinal peptides with GPR43 being proposed to have a regulatory role in the release of peptide YY (PYY) [24] and glucagon-like peptide 1 (GLP-1) [28]. Similarly, GPR120 has been implicated in secretion of cholecystokinin (CCK) and GLP-1 from enteroendocrine cells of the gastrointestinal tract [17, 29, 30]. Several recent studies have identified GPR43 and GPR120 as mediators of the inflammatory response [16, 21, 31] which is consistent with GPR43 expression in immune tissues.

Considered together, these findings suggest a possible role for GPR43 and GPR120 in mediating inflammatory responses and metabolic dysregulation arising in obesity, T2DM and CVD. Recent data has shown that the mRNA expression of both GPR43 and GPR120 is up-regulated by high fat feeding (41% fat; 11 weeks) in mouse adipose tissues [23, 26]. It is now recognised that adipose tissue is not merely a storage organ but rather has the capacity to regulate metabolic health through secretion of adipokines (such as leptin and adiponectin) with increased adiposity also contributing to insulin resistance and metabolic diseases such as T2DM and CVD (reviewed by [32]). However, the effects of these receptors in other metabolically active tissues that are associated with obesity, T2DM and CVD pathophysiology are unknown.

Skeletal muscle is a highly metabolically active tissue, being a major site of systemic lipid metabolism [33] and insulin mediated glucose disposal [34]. Accordingly, skeletal muscle contributes significantly to the maintenance of systemic energy balance. Obesity and T2DM result in perturbed skeletal muscle lipid metabolism, to detrimentally alter substrate partitioning and flux for oxidation [35, 36]. Equally, the liver plays an important role in mediating systemic metabolic health via regulation of glucose and fatty acid metabolism including uptake de novo synthesis, storage and oxidation (reviewed by [37, 38]). Therefore targeting perturbed nutrient metabolism in the liver and skeletal muscle has the capacity to promote euglycaemia and partition fatty acids away from storage. Moreover, aberrant metabolic function in both obesity and type 2 diabetes mellitus contributes to the aetiology and pathogenesis of CVD [39] including cardiac lipotoxicity, glucotoxicity and subsequent myocardial contractile dysfunction. Improving cardiac nutrient metabolism is important in lessening the metabolic stress placed on the heart to reduce morbidity associated with CVD. This suggests that if these receptors regulate metabolic function within the muscle they have the potential to exert profound effects on systemic metabolic health.

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
Cyclophilin	CTGATGGCGAGCCCTTG	TCTGCTGTCTTTGGAACCTTTGTC
GAPDH	AGTTCAACGGCACAGTCAAG	GTGGTGAAGACGCCAGTAGA
GPR120	CCAACCGCATAGGAGAAATC	CAAGCTCAGCGTAAAGCCTCT
GPR43	CACCGAGAACCAATCACCT	GTCATGGGGACGAAAAAGAG

Table 1. 'Real - Time' PCR Primer Sequences. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GPR120, G-protein coupled receptor 120; GPR43 G-protein coupled receptor 43; (Cyclophilin: NM_017101.1, GAPDH: NM_017008.3, GPR120: NM_001047088.1; GPR43: NM_001005877.1).

Given the substantial role of these tissues and the effects of FFAs on systemic metabolic health, we sought to characterise the expression of GPR43 and GPR120 in the liver, skeletal muscle and heart. Moreover we aimed to elucidate the effect of high fat feeding on the tissue abundance of these receptors in skeletal muscle, cardiac muscle and hepatic tissue. It was postulated that diet-induced obesity would up-regulate receptor mRNA expression in these tissues to provide evidence for a possible physiological role in mediating tissue metabolism.

Materials and Methods

Animal Care

Seventeen, six-week old male Sprague Dawley rats (mean initial body weight approximately 178 g) were purchased from Animal Resources Centre (Canning Vale, Australia). Rats were housed within individual cages in an environmentally controlled laboratory (ambient temperature 22-24 °C) with a 12 hour light/dark cycle (7:00 - 19:00). In the week prior to beginning the experiment rats were given free access to food and water. All experimental procedures were approved by the Howard Florey Institute Animal Ethics Committee (AEC 09-050).

Dietary treatments

After 1 week, rats were randomly assigned to receive either a high fat diet (HFD; 22% fat; Specialty Feeds, Glen Forrest, Australia) or a control diet (standard rodent chow; 5% fat; Barastoc Ltd, Melbourne, Australia) (n=8-9/group) for 12 weeks. *Ad libitum* access to food and water was maintained throughout the duration of the study.

Body composition and tissue collection

In week 11, body composition was determined in rats fed a HFD (n = 9) and control diet (n = 4) by dual energy X-ray absorptiometry as previously described [40]. At the conclusion of week 12, individual body weights were recorded for each animal. Rats were then deeply anaesthetised with sodium pentobarbitone (100 mg/kg; Virbac, Peakhurst, Australia) then euthanised via cardiac puncture. Cardiac tissue, hepatic tissue, soleus and extensor digitorum longus (EDL) skeletal muscles

were then removed. All tissue extracts were rapidly frozen on dry ice. Tissue samples were stored at -80°C for subsequent analysis.

'Real - Time' Polymerase Chain Reaction (PCR)

Total RNA was extracted from approximately 15 mg of tissue. Cellular membranes were dissociated using 1000 mg of ceramic/silica beads in TRIzol® Reagent (Invitrogen, Melbourne, Australia) through motorised reciprocation (FastPrep®FP120 cell disruptor; Thermo Electron Corporation, Milford, USA) for 2 x 20 sec bouts. The homogenate was centrifuged (13,000 RPM for 15 min) and the RNA containing supernatant was removed. Homogenate was combined with chloroform (Sigma-Aldrich, St Louis, USA) and total tissue RNA was then extracted using the TRIzol protocol in accordance with manufacturer's instructions with the exception of RNA precipitation which was conducted for a minimum of 2 hours at -20°C in the presence of 10 µl of 5 M sodium chloride. RNA concentration was quantified spectrophotometrically at 260 nm. As the open reading frame of GPR43 is coded by a single exon the extracted RNA was DNase treated using the commercially available RQ1 RNase-free DNase kit (Promega Corporations, Madison, USA) to ensure the sample was free from DNA contaminants. First strand cDNA was then generated from 0.3 µg of template RNA using the commercially available iScript™ cDNA synthesis kit (Bio-Rad Laboratories, Hercules, USA) using random hexamers and oligo dTs as described previously [41]. cDNA was stored at -20°C for subsequent analysis.

'Real - time' PCR was conducted using MyiQ™ single colour 'real-time' PCR detection system (Bio-Rad Laboratories, Hercules, CA) with iQ™ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, USA) as the fluorescent agent. Forward and reverse oligonucleotide primers for the genes of interest were designed using OligoPerfect™ Suite (Invitrogen, Melbourne, Australia) (Table 1). Selective gene homology was confirmed using BLAST analysis (National Centre for Biotechnology Information, Bethesda, USA). To compensate for variations in RNA input amounts and reverse transcriptase efficiency mRNA abundance of the genes of interest were normalised to housekeeping genes, GAPDH (heart and liver) and cyclophilin (skeletal muscle). 'Real - time' PCR reactions were run for 50 cycles of 95°C for 15 sec and 60°C for 60 sec. Relative changes in mRNA abundance was quantified using the 2^{-ΔΔCT} method as previously detailed [42] and reported in

Fig. 1. Skeletal muscle mRNA abundance of GPR43 and GPR120 in rats fed a control diet (5% fat) compared to high fat diet (22% fat) for 12 weeks. Data expressed in arbitrary units normalised to cyclophilin as mean $\pm$ SEM. n = 7-8/group. Opened bars represent control diet; closed bars represent high fat diet. A: GPR43 expression in soleus muscle; B: GPR43 expression in EDL muscle; C: GPR120 expression in soleus muscle; D: GPR120 expression in EDL muscle. * $p \leq 0.05$ high fat diet compared to control.

Fig. 2. Hepatic mRNA abundance of GPR43 and GPR120 in rats fed a control diet (5% fat) compared to high fat diet (22% fat) for 12 weeks. Data expressed in arbitrary units normalised to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as mean $\pm$ SEM. n = 8-9/group. Opened bars represent control diet; closed bars represent high fat diet. A: GPR43 expression in hepatic tissue; B: GPR120 expression in hepatic tissue. * $p \leq 0.05$ high fat diet compared to control diet.

arbitrary units. C_T values for GAPDH and cyclophilin were not altered by dietary intervention.

Statistical Analysis

All statistical analyses were conducted using PASW statistics version 18 (SPSS Inc., Chicago, USA). All data is reported as mean $\pm$ SEM. Independent two tailed t-tests were performed on the samples to determine between group differences. Statistical significance was accepted at $p \leq 0.05$.

Results

Animal body weight and fat mass

Rats maintained on a HFD for 12 weeks weighed significantly more compared to control (635 ± 21 g and

548 ± 37 g, respectively; $p \leq 0.05$). The percentage of global body fat mass was also seen to be significantly increased in the high fat fed rats compared to control (24.3 ± 1.7 % and 12.9 ± 1.5 %, respectively; $p \leq 0.05$).

Skeletal muscle expression of GPR43 and GPR120

Interestingly, 12 weeks of high fat-feeding in rats resulted in significant increase in GPR43 mRNA abundance in soleus and EDL skeletal muscle compared to control ($p \leq 0.05$; Fig. 1). GPR120 mRNA expression was also significantly increased subsequent to a HFD in the EDL skeletal muscle compared to control ($p \leq 0.05$; Fig. 1). However, the HFD did not alter GPR120 mRNA

Fig. 3. Cardiac tissue mRNA abundance of GPR43 and GPR120 in rats fed a control diet (5% fat) compared to high fat diet (22% fat) for 12 weeks. Data expressed in arbitrary units normalised to glyceraldehyde 3-phosphatase dehydrogenase (GAPDH) as mean $\pm$ SEM. n = 8-9/group. Opened bars represent control diet; closed bars represent high fat diet. A. GPR43 expression in cardiac tissue; B. GPR120 expression in cardiac tissue. * $p \leq 0.05$ high fat diet compared to control diet.

expression from the control diet in soleus muscle (Fig. 1).

Hepatic receptor expression of GPR43 and GPR120

Hepatic GPR43 expression was significantly up-regulated in the rats which received the HFD for 12 weeks when compared to control ($p \leq 0.05$; Fig. 2). However, hepatic GPR120 mRNA abundance was unchanged by diet (Fig. 2).

Cardiac receptor expression of GPR43 and GPR120

The HFD resulted in differential dietary regulation of GPR43 and GPR120 in the heart. The abundance of GPR120 mRNA in cardiac tissue was significantly up-regulated in rats fed the HFD compared to rats fed the control diet ($p \leq 0.05$; Fig. 3). Conversely, cardiac GPR43 mRNA expression was not altered by the dietary intervention (Fig. 3).

Discussion

GPR43 and GPR120 are novel GPCRs which have recently been shown to be activated by FFA [13-17]. These receptors modulate parameters of metabolic homeostasis such as adipogenesis and release of regulatory peptides GLP-1, PYY and CCK [23, 24, 26-28]. This indicates that selective manipulation of GPR43 and GPR120 could be a possible strategy for attenuating increases in fat mass and regulating systemic parameters of metabolic health such as glucose homeostasis and satiety.

Here we have provided additional data that GPR43 and GPR120 RNA transcripts are present in skeletal muscle, liver and heart. Further, we have demonstrated

that there is a differential regulation of GPR43 and GPR120 tissue specific expression in the presence of a high fat environment *in vivo*, in skeletal muscle, liver and heart tissue. Our findings support a possible role for GPR43 and GPR120 in selectively mediating metabolic processes in these tissues.

In the current study, we have identified GPR43 and GPR120 mRNA in skeletal muscle, cardiac muscle and hepatic tissue. These findings are consistent with previous research showing GPR43 and GPR120 mRNA to be expressed in these tissues [17, 23, 26] however, alternative research does not support the expression of GPR43 and GPR120 mRNA in these tissues [16, 22, 30]. The reason for this discrepancy is unclear, however the low mRNA abundance of GPR43 and GPR120 in these tissues may help to explain the conflicting data.

Importantly, we characterised a significant up-regulation of GPR43 mRNA expression in soleus, EDL skeletal muscle and the liver in rats fed a HFD for 12 weeks. However the dietary intervention did not alter the mRNA abundance of GPR43 in the heart. The reason for this divergent regulation is unclear and does not discount a possible role for GPR43 in mediating cardiac metabolism. Further research is required to clarify these effects.

Bjursell et al. [43] have shown GPR43^{-/-} mice to have reduced fat mass and increased lean muscle mass compared to wild-type mice fed a HFD despite having increased energy intake. This study also reported favourable effects such as increased plasma adiponectin concentration, decreased cholesterol and hepatic triglyceride content. Similarly, glucose control was improved as indicated by a lowered homeostatic model assessment score in the GPR43 deficient mice fed a HFD compared to wild-type [43]. These findings are indicative of a role for GPR43 in mediating systemic metabolic homeostasis. GPR43 has been demonstrated to exhibit

dual coupling to both Gi and Gq signalling pathways to mediate intracellular signalling [14]. GPR43 coupling increases intracellular calcium concentration and decreases intracellular cyclic adenosine monophosphate (cAMP) concentrations [14, 22, 44]. GPR43 stimulation is associated with phosphorylation and activation of mitogen activated protein kinases (MAPK) including extracellular signal-regulated kinase 1/2 (ERK1/2; also designated p44/p42 ERK) and p38 [14, 31, 44]. In the skeletal muscle, activation of the ERK1/2 pathway by free fatty acids has been associated with nuclear factor- κ B (NF κ B) activation [45, 46] and subsequent down-regulation of PGC1 α [46]. PGC1 α induces expression of many genes involved in the regulation of the cellular energy state [47, 48] and has been shown to promote mitochondrial biogenesis and function [49, 50], fatty acid oxidation [51-53] and muscle fibre type plasticity. Thus, PGC1 α supports a more oxidative phenotype [54, 55]. Green et al. [45] also propose that ERK1/2 activation of NF κ B may promote skeletal muscle inflammation to the detriment of metabolic homeostasis. Indeed, NF κ B activity has been shown to be increased in skeletal muscle from obese and obese type 2 diabetic subjects compared to lean counterparts [56]. Studies by Sinha et al. [57] further substantiate this, showing that a peptide inhibitor of NF κ B nuclear translocation attenuates saturated fatty acid induced insulin resistance in L6 skeletal muscle myotubes. This suggests that up-regulation of GPR43 in skeletal muscle may impair substrate partitioning and flux for oxidation.

GPR43 activation of p38 MAPK also has the potential to influence skeletal muscle metabolism of glucose and fatty acids [58-60]. However the role of p38 MAPK in the skeletal muscle is less clear. Under homeostatic conditions, research has shown that activation of p38 α and p38 β isoforms is important for insulin stimulated glucose uptake [59, 60]. However prolonged activation of p38 was shown to down-regulate insulin stimulated glucose transport in L6 myotubes [58]. This suggests dysregulation of this pathway may contribute to the insulin resistant phenotype seen in the obese and diabetic disease states. In support of this, Ho et al. [61] reported positive regulation of basal skeletal muscle glucose uptake but negative regulation of skeletal muscle glucose uptake in response to increased cellular energetic stress in L6 myotubes over-expressing the p38 γ isoform. In view of these findings, it is possible that the up-regulation of skeletal muscle GPR43 observed in current study may be associated with potentiation of aberrant fatty acid metabolism and insulin resistance

within this tissue. Such physiological changes favour the development of obesity, T2DM and associated comorbidities.

GPR43 mRNA was also up-regulated in the liver subsequent to diet-induced obesity in the current study. The ERK1/2 pathway appears to be an important modulator of hepatic lipogenesis, with ERK1/2 activation being associated with insulin-induced up-regulation and leptin-induced down-regulation of stearoyl-CoA desaturase 1 (SCD1) expression [62]. SCD1 is primarily involved in the desaturation of palmitoyl-CoA and stearoyl-CoA to their monounsaturated palmitoyl-CoA and oleoyl-CoA derivatives, which are important substrates in *de novo* lipogenesis (reviewed by [63]). Hyperinsulinaemia, as observed in pre- and overt diabetes, may therefore be associated with induction of SCD1. Consistent with this, Seo et al. [64] show that obese, hyperinsulinaemic Otsuka Long-Evans Tokushima Fatty rats have significantly up-regulated hepatic SCD1 expression compared to control rats. ERK1/2 phosphorylation also appears to promote the activity sterol regulatory element binding protein (SREBP) [65], with Kotzka et al. [66] providing evidence for this in hepatic hepG2 cells. SREBP regulates the transcription of a number of genes involved in promoting hepatic lipid synthesis, including SCD1 (reviewed by [67]). Induction of lipogenic genes provides a plausible mechanism whereby up-regulation of hepatic GPR43 may contribute to the development of dyslipidaemia through augmented hepatic lipid synthesis.

The effects of diet-induced obesity on GPR120 receptor expression in rats following 12 weeks high fat feeding was again tissue specific. Here, mRNA abundance of GPR120 was observed to increase in the EDL skeletal muscle and cardiac muscle from rats fed the HFD when compared to control animals. The mRNA abundance of the GPR120 receptor was unaltered by the HFD in the liver and soleus skeletal muscle. The reason for the divergent regulatory effect in these skeletal muscles is unclear, however is likely related to the different composition of myofibres within these muscles. EDL muscle is primarily composed of glycolytic myofibres, whilst soleus muscle is composed primarily of oxidative myofibres [68, 69]. This has implications for subsequent nutrient oxidation with EDL muscle exhibiting a lower oxidative capacity and therefore a greater capacity to oxidise glucose over fatty acids [70]. This is indicative of a distinct role for GPR120 in EDL compared to soleus skeletal muscle and may imply that GPR120 is a more potent regulator of glucose than

lipid oxidation. Concordant with this, Oh et al. [16] demonstrated that GPR120^{-/-} mice became insulin resistant, with animals manifesting hyperinsulinaemia and decreased insulin stimulated glucose disposal. However, the specific functional consequences of the selective regulation of GPR120 expression in the different skeletal muscle types remains to be characterised.

Previous research has determined that GPR120 couples to the Gq and β -arrestin2 to modulate cell signalling pathways [16, 71]. GPR120 has been shown to increase intracellular calcium content and ERK1/2 phosphorylation when activated [16, 17, 71]. Consistent with the aforementioned effects of ERK1/2 activation on skeletal muscle insulin sensitivity and oxidative function [45, 46, 57], it is reasonable to assume that GPR120 activation in skeletal muscle may exert a pro-inflammatory effect. This suggests that GPR120 activation in the skeletal muscle may promote insulin resistance and decrease fatty acid oxidation through the activation of the NF κ B pathway. However this is inconsistent with previous findings showing GPR120 activation to have anti-inflammatory effects in a number of cell types including adipocytes and macrophages [16]. It is also possible that ERK1/2 phosphorylation mediated by β -arrestin2 results in dissociable outcomes when compared to Gq coupling. β -arrestin2 activation of ERK1/2 has been shown to occur more gradually, last longer and associate with receptor internalisation in place of second messenger signalling (reviewed by [72]). This suggests that the effects elicited by ERK1/2 activation may differ. The finding that GPR120 activation by LCFA results in GPR120 being sequestered to the cytosol [16] is suggested to be consistent with distinct effects. However, subsequent functional outcomes of GPR120 activation on ERK1/2 signalling in skeletal muscle are unknown.

The novel finding that GPR120 is up-regulated in heart tissue is also supportive of a functional role for this receptor in cardiac function. ERK1/2 activation in cardiac tissue has been associated with induction of hypertrophic gene profile and subsequent cardiac hypertrophy [73, 74]. This relationship is especially apparent in response

to pathophysiological stimuli such as pressure overload and has been reviewed extensively elsewhere [75]. Therefore it is possible that in cardiac tissue, GPR120 may activate ERK1/2 to promote cardiac hypertrophy. Obesity is associated with hypertension and the development of cardiac hypertrophy [76-78]. Non-compensatory cardiac hypertrophy is positively correlated to adverse cardiovascular events and death [79]. Thus in the presence of metabolic diseases such as obesity, GPR120 up-regulation may be partially responsible for cardiac hypertrophy and subsequent negative cardiovascular outcomes. If correct this has the potential to increase morbidity and mortality associated with CVD and therefore this avenue warrants further investigation.

In summary, this study has further characterised the distribution of GPR43 and GPR120 receptors in skeletal muscle, cardiac muscle and the liver. In addition, this study has demonstrated that the abundance of GPR43 and GPR120 is regulated in a tissue specific manner by diet-induced obesity in rats. When considered in conjunction with the current knowledge of the functions of these receptors we propose that both GPR43 and GPR120 may have important regulatory roles in the respective tissues. This has implications with reference to the alleviation of the obesity, T2DM and CVD. We postulate that selective manipulation of GPR43 and GPR120 in the heart, liver and skeletal muscle has therapeutic potential in ameliorating metabolic disturbances associated with these conditions. Therefore, qualifying the functional consequences of GPR43 and GPR120 in these metabolically active tissues is highly pertinent.

Acknowledgements

LMC was supported by a scholarship (PB 10M 5472) from the National Heart Foundation of Australia. This research was supported by a National Heart Foundation of Australia Grant-in-Aid (G07M3227) (MLM).

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