Exploring the relationship between gut microbiota and exercise: short-chain fatty acids and their role in metabolism

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ABSTRACT
The human body is host to a multitude of bacteria, fungi, viruses and other species in the intestine, collectively known as the microbiota. Dietary carbohydrates which bypass digestion and absorption are broken down and fermented by the microbiota to produce short-chain fatty acids (SCFAs). Previous research has established the role of SCFAs in the control of human metabolic pathways. In this review, we evaluate SCFAs as a metabolic regulator and how they might improve endurance performance in athletes. By looking at research conducted in animal models, we identify several pathways downstream of SCFAs, either directly modulating metabolic pathways through second messenger pathways or through neuronal pathways, that contribute to energy utilisation. These pathways contribute to efficient energy metabolism and are thus key to maximising substrate utilisation in endurance exercise. Future research may prove the usefulness of targeted dietary interventions allowing athletes to maximise their performance in competition.

INTRODUCTION
The human body is composed of a diverse array of microorganisms, including bacteria, archaea, viruses and fungi. These organisms are collectively known as the microbiota and reside throughout and on the body, including in the lungs, oral mucosa, uterus and on the skin. A key location for the microbiota is the gastrointestinal tract, in particular the large intestine, where it is calculated that there are up to 100 trillion (1×10^{14}) microbes. The role of the microbiota in human physiology is becoming increasingly understood, from its role in inflammatory diseases and hypersensitivity to the importance of a diverse microbiota for mental health.

The composition and function of the microbiota vary among different populations. This is particularly true of elite athletes and obese individuals compared with other human subjects. How the microbiota impacts body composition is understood to be through its influences on metabolic pathways (including liver and adipose tissue), in addition to energy extraction from diet and hormone signalling in the gut.

Additionally, in animal experiments, germ-free (GF) mice were significantly worse at endurance exercise compared with mice that had Bacteroides fragilis colonisation of the colon. This poses the question: what contribution does the gut microbiota have on aerobic activity in athletes? One of the metabolites that may explain the role of the microbiota on exercise is short-chain fatty acids (SCFAs).

Short-chain fatty acids
Some dietary carbohydrates which bypass digestion are broken down and fermented by bacteria that make up the microbiota in the large intestine. These insoluble carbohydrates break down into three of the most common SCFAs—acetate, propionate and butyrate, composed of hydrocarbon chains of 2, 3 and 4 carbon chains, respectively. Importantly, the amount of SCFAs is not only regulated by the type of bacteria, but also by food in the intestine. In that way, the composition and quantity of SCFAs can readily be modified through dietary interventions, making it a target for athletes. Our current understanding is SCFAs mediate the interaction between diet, microbiota and the host, primarily through activation of G protein-coupled receptors (GPCRs).
Fatty acid GPCRs

SCFAs are not restricted to the lumen of the gut as they can be found systemically in the blood, pancreas and brain.\textsuperscript{15} GPR41 and GPR43 (also known as FFAR3 and FFAR2, respectively) are a small family of GPCRs of which the ligand is colonic SCFAs.\textsuperscript{16}

There is evidence that the effects of SCFAs in health and disease are diverse\textsuperscript{17–19} and the expression of these receptors throughout the body supports the hypothesis that SCFAs are essential in physiological signalling and control of metabolism throughout multiple organs. Therefore, regulation and refinement of host metabolism are necessary for exercise, in particular endurance exercise.\textsuperscript{20}

The recent paper by Frampton \textit{et al}\textsuperscript{21} is an excellent review on SCFAs and metabolism. The authors review the wider scope of effects on skeletal muscle, such as the effect on muscle mass and muscle phenotype. Frampton \textit{et al}\textsuperscript{21} analyse the indirect effects of SCFAs, such as the effect on inflammation, and the implications on skeletal muscle, but they do not go further to link this to aerobic performance. The authors review metabolic pathways, for example, carbohydrate metabolism without further explanation about how this would have an effect on skeletal muscle output, and importantly, without any mention of aerobic exercise performance. This recent review\textsuperscript{21} is in line with analysis of SCFAs in metabolism and cardiovascular health\textsuperscript{17} and in inflammation, glucose and lipid metabolism.\textsuperscript{22}

In contrast, our literature review is unique in the context of aerobic exercise performance, analysing the effects of SCFAs on aerobic capacity of skeletal muscle only.

Exercise

In professional athletes, and also amateur sporting populations, the ability to perform at consistently high levels during competition and training is vital. Endurance activities, such as marathon running, triathlons or cross-country skiing, are multifaceted disciplines relying on effective and efficient energy metabolism.

SCFAs can be used directly as an additional substrate for metabolism.\textsuperscript{23} These are predominantly metabolised by enterocytes of the intestine and liver.\textsuperscript{24} SCFAs are independent of carnitine for uptake and result in intramitochondrial activation to acyl-CoA.\textsuperscript{25} They also act as dedicated signalling molecules modulating the metabolism of other substrates.\textsuperscript{15}

While it has been shown that exercise conveys benefit for the health of gut bacteria, it has not been shown whether the converse is true—whether the health of the gut bacteria has an impact on performance. The exact role of the microbiota and the metabolic function and the effect of SCFAs in the body can provide a greater comprehension whether improving the health and diversity of the microbiota may provide a competitive advantage in athletes.

While the methodology of the included articles varies significantly, there are common themes in the research questions. First, we will explore the work by Arora \textit{et al}\textsuperscript{26} De Vadder \textit{et al}\textsuperscript{27} and Korecka \textit{et al}\textsuperscript{28} which investigate the effects of microbiota-derived metabolites in the enteroendocrine system. All three papers conducted experiments in murine models, except for Korecka \textit{et al}\textsuperscript{28} who additionally used an in vitro cell line. The work by Kimura \textit{et al}\textsuperscript{29} Wichmann \textit{et al}\textsuperscript{30} and Zadeh-Tahmasebi \textit{et al}\textsuperscript{31} explore the role of intestinal metabolites in the plasma and thus demonstrate to us the potential for system-wide effects of SCFAs. Finally, Barton \textit{et al}\textsuperscript{32} the only study to use human subjects, corroborate earlier research by exploring the metabolic phenotyping and biodiversity of the microbiota in elite athletes. For an overview of the articles methods and key findings, please see the online supplemental appendix 1.

The role of SCFAs in the enteroendocrine system

Arora \textit{et al}\textsuperscript{26} set out to identify whether dietary supplementation of fermentable soluble flaxseed fibre affects SCFA production in mice colons and how the SCFAs from this fermentation and downstream activation of the GPR41 receptor affects the enteroendocrine function within the colon and ileum. The authors deduced the transcriptome of enteroendocrine cells in both the ileum and colon was upregulated when supplemented with flaxseed fibre in a mouse model, and this was under the control of SCFAs. The researchers identified that supplementing the mouse diet with flaxseed fibre, which in turn increased the availability of SCFAs, also observed that the transcriptome of enteroendocrine cells in both the ileum and colon was upregulated. Of note, genes associated with cell division and protein catabolism increased.

De Vadder \textit{et al}\textsuperscript{27} demonstrate that intestinal metabolic pathways, in particular, upregulation of gluconeogenesis is, in part, under the control of SCFAs. First, the authors demonstrated, through Caco-2 cell models, that SCFAs from this fermentation and downstream activation of the GPR41 receptor affects the enteroendocrine function within the colon and ileum. The authors showed that the 24-hour incubation with pronipionate did not impact the expression of G6PC, PCK1 and MUT in Caco-2 cells. However, the appearance of G6PC and PCK1 was increased twofold to threefold in cells incubated with butyrate showing that butyrate, but not pronipionate, is able to directly increase gene expression related to intestinal gluconeogenesis (p.67).

De Vadder \textit{et al}\textsuperscript{27} then quantified intestinal glucose production in rat models, fed propionate, butyrate or fructo-oligosacharides (FOS), for 2 weeks. Resulting in glucose-specific activity being “lower in the portal vein than in the artery” (p.86), demonstrating that the intestine had released unlabelled glucose (synthesised through gluconeogenesis). The authors demonstrated also that 23% of all systematic glucose was derived from intestinal gluconeogenesis.
De Vadder et al\textsuperscript{7} went on to show that propionate was the greatest inducer of intestinal gluconeogenesis and that the mechanism of this was through a gut–brain communication mechanism leading to intestinal gluconeogenesis, rather than direct induction. Through capsacin-mediated perportal deafferentation, the authors demonstrated that G6Pase activity and PCK1 protein expression were not increased in the intestine compared with controls. However, dietary butyrate and FOS did increase G6Pase activity and PCK1 expression in the treated rat group. This shows that there is a role of the periportal nervous system in transducing the effects of intestinal gluconeogenesis (and portal glucose sensing) from propionate.

Unlike Arora et al\textsuperscript{26} who demonstrated that there are localised effects in the gastrointestinal tract through gene expression, De Vadder et al\textsuperscript{7} show that localised control of metabolic pathways in the gastrointestinal tract is influenced through more comprehensive pathways, in particular a neuronal pathway connecting the gut and brain.

Korecka et al\textsuperscript{28} explore the underlying mechanism of intestinal bacterial influencing metabolism, in particular by evaluating the role of angiopoietin-like protein 4 (ANGPTL4) in the gastrointestinal tract. The authors, using an in vitro cell model of three different cell lines, identified that ANGPTL4 is induced by the butyrate, and that can regulate host metabolism independent from PPAR-\(\gamma\). They demonstrated butyrate could induce expression of ANGPTL4 mRNA in intestinal epithelial cells both in vitro and in vivo. They identified that when treating the cells with butyrate, the level of PPAR-\(\gamma\) was lower. They also found that ANGPTL4 expression in the ileum and colonic cells increased when exposed to \textit{Clostridium tyrobutyricum} bacteria, confirming the effect that bacteria-produced SCFAs has on the expression of ANGPTL4.

Korecka et al\textsuperscript{28} further corroborate the role of SCFAs influencing metabolic pathways in the gastrointestinal tract. Unlike Arora et al\textsuperscript{26} and De Vadder et al\textsuperscript{7} who demonstrate that gastrointestinal, metabolic pathways are influenced by SCFAs through the GPR41 and GPR43 family of receptors, Korecka et al\textsuperscript{28} show that it is not just this family of receptors which contributes to metabolic changes, but the ANGPTL4 receptor too.

**System-wide effects of SCFAs**

Kimura et al\textsuperscript{29} researched the role of the GPR43 (FFAR3) receptor in the regulation of energy balance, identifying the role of the receptor in insulin signalling in adipose tissue. The authors demonstrated that mice deficient in GPR43 receptors through gene knockout (GPR43 \(-/-\)), become obese on a regular diet. However, by using promoter-driven transgenic mice that overexpressed GPR43 in adipose tissue, they identified that the mice remained lean even when fed a high-fat diet. They concluded that “GPR43 receptor acts as a sensor for excess dietary energy, thereby controlling body energy utilisation while maintaining metabolic homeostasis” (p.2). The researchers identified in transgenic aP2-GPR43TG mice, overexpressing GPR43, that insulin sensitivity was improved. They identified insulin-induced Akt phosphorylation in white adipose tissue but not in muscle or liver tissue. Kimura et al\textsuperscript{29} subsequently administered exogenous acetate and found this suppressed insulin signalling in adipocytes of wild-type mice. In the GPR43 deficient mice, on the other hand, this effect was unseen. They concluded, the activation of the GPR43 receptor by SCFAs suppress insulin pathways in adipocytes, leading to the inhibition of fat accumulation in adipose tissue. The authors showed that the influence of SCFAs on GPR43 receptors is wider than the gastrointestinal tract. They also demonstrated the importance of the GPR43 in the regulation of insulin gesturing in adipocytes. The delicate balance of storage versus catabolism of lipids is an essential regulator in host energy regulation and is essential for efficient use of energy substrates during exercise.

Wichmann et al\textsuperscript{30} looked at how the microbiota can modulate energy availability in the colon, through glucagon-like peptide 1 (GLP-1). Using mouse models, the authors compared portal vein levels of GLP-1 in GF mice to conventionally raised mice. The researchers identified that in mice that have an absence of intestinal microbiota, there is an upregulation of GLP-1 expression levels to slow intestinal emptying. Also observed was that the absence of microbiobially produced SCFAs in a GF mouse colon results in significantly higher plasma GLP-1 levels compared with conventionally raised mice.

Wichmann et al\textsuperscript{30} measured caecal SCFA concentration as a measure of energy availability. They transplanted unfractionated microbiota from a conventionally raised donor into GF mice. \textit{Escherichia coli} resulted in a small increase in acetate, but overall SCFAs were unchanged, as were an expression of Gcg and GLP-1 positive cells. Thus, it can be elucidated that the elevated GLP-1 expression is due to increased proglucagon gene expression in the colon. On the other hand, \textit{B. thetaiotaomicron} levels resulted in increased acetate and propionate, a four-fold increase in total SCFAs, and an associated 2.5-fold decrease in Gcg and 1.7 decreases in GLP-1 positive cells. In sum, Wichmann et al\textsuperscript{30} show that SCFA concentration in the gut modulates GLP-1, an important metabolic regulator in the plasma.

Using male Sprague-Dawley rats, Zadeh-Tahmasebi et al\textsuperscript{31} conducted experiments to identify the metabolic changes in rodents exposed to SCFAs. The authors demonstrated that glucose production is modulated through a GPR43 negative feedback loop, dependent on neuronal networks, highlighting the role of intestinal metabolites in glucose homeostasis. To identify the underlying mechanism of propionate sensing, the researchers removed the ileal mucosa, lysed and subjected the tissue western blot analysis of GPR43. A positive result was identified, with GPR43 expression being shown in the ileal mucosal tissue. LV-FFAR shRNA was injected into ileum...
3 days before the clamp studies. They demonstrated that without GPR43 expression, glucose infusion rate and glucose production remained the same, showing that propionate sensing is dependent on the activation of GPR43.

The authors31 focused on identifying how the production of glucose is modulated by the gut bacteria and its metabolites. Similarly, Wichmann et al30 and Zadeh-Tahmasebi et al31 identified the role of GLP-1, which regulates a neuronal network. This is not dissimilar to the work of De Vadder et al27 who identified that localised control of metabolic pathways in the gastrointestinal tract is influenced through more extensive pathways, in particular a neuronal pathway connecting the gut and the brain. Zadeh-Tahmasebi et al31 highlighted that neuronal networks influence glucose production.

**Relationship between microbiota and exercise**

Barton et al32 analysed metabolic phenotyping and functional metagenomics of the gut microbiome of 40 international rugby union players compared with age-matched healthy controls in two groups, body mass index (BMI) less than 25 and BMI greater than 25 to account for variability in body composition of rugby union players.

The diversity of microbiota was significantly higher in the athletes compared with both high BMI and low BMI human controls. In particular, there was an increase of the genus Akkermansia among the elite athletes. As a result of the increased biodiversity, the SCFA levels in faeces, as measured by “gas chromatography-mass spectrometry (GC-MS)” showed significantly higher levels of acetate (p<0.001), propionate (p<0.001), butyrate (p<0.001) and valerate (p=0.011) in athletes relative to controls” (p.628).32 The authors accounted for dietary intake, primarily overall energy intake, sugar, starch, total carbohydrate, fat, protein and fibre intake. There was an important correlation between concentrations of propionate and protein intake. In addition, butyrate had a strong relation to the intake of dietary fibre.

Ninety-eight metabolic passageways differed between the three cohorts. In the elite athlete cohort, it was observed that there was the highest abundance of upregulated paths—29 out of 34 of these were involved in upregulation of metabolism. This included carbohydrate biosynthesis, cofactor biosynthesis and genes involved in energy metabolism. There was a statistically significant correlation between the metabolic pathways to GC-MS-identified faecal SCFA concentrations. Barton et al28 were the only researchers who used humans as their experimental subjects. They corroborate work done in animal models, however, by showing upregulation of a significant number of genes associated with SCFAs.

Several bodies of research have identified that exercise has positive effects on the composition and diversity of the microbiota.33 34 In animal models, microbiota and associated metabolites influence metabolic pathways in the gastrointestinal tract, the adipose tissue and the metabolites, specifically SCFAs, are found systemically. In this way, we can consider the possibility that SCFAs may provide an alternative fuel for endurance exercise through direct utilisation in the skeletal muscle and by liberating other carbohydrate substrates through modifying metabolism.

**Endurance exercise**

The demands of endurance exercise from a metabolic standpoint, particularly at elite levels, are significant.35 Liver glycogen supplements muscle glycogen as an energy reserve that can be used in prolonged exercise. To put this in perspective, the total body glycogen store is estimated to be 103 mol of adenosine triphosphate (ATP) at best, which is insufficient to provide the 150 mol of ATP needed for a marathon lasting >2 hours.36

It is well documented that after particularly long-endurance events, there is a marked caloric deficit,37 depletion of muscle and liver glycogen stores and generation of ketone bodies.38 We can infer, then, that for maximal performance, metabolic flexibility is essential.39 40

It is known that SCFAs derived from the microbiota are directly used in skeletal muscle.40 First, the SCFA receptor GPR42 is expressed in muscle,41 which suggests there may be a role for SCFA signalling within myocytes. However, there have been limited studies evaluating SCFAs on muscle metabolism. In experiments on obese rats, 6 months of acetate injections (5.2 mg/kg of body weight) increased gene expression of oxidative phosphorylation and glucose metabolic pathways, including the genes encoding Glut4, myoglobin and AMPK.42

**SCFAs in the nervous system**

We have seen from the work of De Vadder et al27 that there is a gut–brain communication pathway reliant on SCFAs. Additional research has shown us that there are receptors for SCFAs throughout the nervous system.43 There is an expression of the SCFA receptor GPR41 in both autonomic and somatic sensory ganglia. This is particularly important in host metabolism in exercise, as a regulator of energy expenditure is the sympathetic nervous system. The identification of GPR41 receptors in the superior cervical ganglion suggests that GPR41 could be a candidate for an autonomic nervous system sensing of SCFAs originating from dietary fibres having a downstream effect on metabolism through nutrient sensing.44

Early research conducted in cultured superior cervical neurons showed us that GPR41 activation by SCFAs had been shown to release norepinephrine,44 suggesting that SCFAs possess the ability to enhance sympathetic activity. This may be of significance in the context of exercise performance, as afferent neural information from muscles under load establishes a pattern of sympathetic-adrenal activity according to the relative intensity of the exercise.45

**Metabolism**

The gut microbiota impacts nutrient intake and energy regulation and a microbiota dysbiosis can influence the
development of obesity, insulin resistance and diabetes. In human subjects consuming a ‘typical western diet’, it was found that microbiologically produced SCFAs contributed around 10% of total energy requirements, and the contribution is expected to be higher for humans consuming high-fibre diets and for exclusively plant-eating species.

**ANGPTL4 expression**

The angiopoietin-like proteins, a family of secreted proteins, have a role in energy metabolism. They are named because they share tertiary structural domains with angiopoietins. ANGPTL4 has been shown in previous research to be involved in upregulating lipoprotein lipase (LPL) activity, which is vital in metabolism, as LPL breaks down triglycerides into free fatty acids which can be used by metabolising tissue, through beta-oxidation, including in muscle tissue.

Korecka et al. showed that ANGPTL4 expression is induced by the SCFA butyrate in the gastrointestinal tract. While their work does not demonstrate to us whether butyrate-induced ANGPTL4 is available in skeletal muscle, other research by Catoire et al. concluded that during endurance exercise, induction of ANGPTL4 in non-exercising muscle reduces local fatty acid uptake, presumably to prevent fat overload, while directing fatty acids to the active skeletal muscle as fuel. Another limitation of the Korecka research was that butyrate was administered via oral gavage to GF mice. While this may show whether a form of exogenous SCFA may convey a benefit, an improved method could also be to increase SCFAs through modification of diet. Further research is warranted to identify whether the ANGPTL4 expressed by ileum and colonic epithelial cells has an impact on skeletal muscle tissue and to determine if effects are seen through a dietary modification to upregulate SCFAs.

**GLP-1 signalling**

The effect of GLP-1 on glucose metabolism is understood. However, exposure to SCFAs, through the work of Wichmann et al., demonstrates that there are lower levels of GLP-1. Through the dogma of GLP-1 signalling in insulin secretion, we can deduce there is an associated decrease in insulin while glucagon increased. This resulted in increased glucose uptake in skeletal muscle and increased glucose production in the liver. These are key to increasing glucose availability for skeletal muscle and thus prolonging the time to exhaustion. The expression of the GPR41 receptor is present in enteroendocrine cells in the beta cells of the pancreas, which identifies that pancreatic release of insulin is not just under the control of circulating glucose and long-chain fatty acid levels, but also under the influence of molecules from the colon. As such, these receptors are being considered as novel drug targets for the treatment of metabolic syndrome because they are activated by fatty acids. The role of SCFAs in glucose homeostasis in individuals without diabetes, in particular athletes, may be important for ensuring energy demands are met during competition.

**Implications for athletes**

In animal experiments, GF mice were shown to perform worse at endurance exercise than mice with *B. fragilis* colonisation of the colon. This corroborates the role of SCFAs in metabolic pathways. In animal models, there is a link between SCFAs, gene expression in the gut, ANGPTL4 expression and GLP-1 expression. Other research shows the role of intestinal metabolites in mitochondrial function. All such metabolic contributions show a promising avenue of research for how the gut can be fine-tuned in athletes to maximise performance. Furthermore, there may be an important cluster of pathways modulated by intestinal metabolites that thus far have been neglected. Optimum nutrition is essential for peak athlete performance, and we can see that going forward more research into specific dietary supplements that might target bacteria known to generate SCFAs and thus likely improve exercise performance is warranted.

**CONCLUSION**

The majority of research surrounding metabolites produced in the gastrointestinal tract has focused on outcomes concerning metabolic syndrome or other disease states. In this review, by concentrating on a relatively small number of articles that looked at pathways downstream of SCFAs, several have been identified that may be physiologically implicated in exercise performance. These data, when put together, show a highly promising avenue of research for how the gut can be fine-tuned in athletes to maximise performance in and outside competition. However, the lack of human trials or a consensus in methodology for exploring SCFA pathways is extremely limiting at this point. Furthermore, the lack of experimentation in athlete populations makes recommendations challenging and preliminary.

However, this is an exciting area of research that would benefit from further investigation. One possibility is to compare time with exhaustion in an animal model after exogenous SCFAs. Another could be to measure the plasma concentrations of SCFAs in athletes in competition to evaluate how performance is related.

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REFERENCES


### Appendix 1

<table>
<thead>
<tr>
<th>Title</th>
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<td>Microbial Fermentation Of Flaxseed Fibres Modulates The Transcriptome Of GPR41-Expressing Enteroendocrine Cells And Protects Mice Against Diet-Induced Obesity.</td>
<td>Arora, Tulika Rudenko, Olga Egerod, Kristoffer Lihme, Husted, Anna Sofie Kovatcheva-Datchary, Petia Akrani, Rozita Kristensen, Mette Schwartz, Thue W Bäckhed, Fredrik</td>
<td>2018</td>
<td>Mouse</td>
<td>GPR41 promoter controlled red fluorescent protein in mice fed 4 different diets: normal chow, high fat diet, high fat diet with 10% non-fermentable fibre cellulose and high fat diet with 10% fermentable flaxseed fiber. All diets were conducted for 12 weeks. Evaluation of: microbiota changes enteroendocrine cell transcriptome in ileum and colon. Additionally, the authors measured body weight and fat mass, energy expenditure, food and water intake during exercise. SCFAs and organic acids were measured by gas chromatography. GPR41 controlled RFP mice. Randomly assigned to the aforementioned 4 diets. The ileum and colon tissue was harvested, used to create a single cell suspension for RNA extraction to evaluate the transcriptome.</td>
<td>Supplementation of flaxseed fibres increases the abundance of caecal Akkermansia and Bifidobacterium. A high fat with the addition of flaxseed fibre restored butyrate level to comparable of that in mice fed normal chow. Measurements of acetate and propionate were unaffected by soluble fibre. Flaxseed supplementation alters GPR41 expression.</td>
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<td>The Microbiome Of Professional Athletes Differs From That Of More Sedentary Subjects In Composition And Particularly At The Functional Metabolic Level</td>
<td>Barton, Wikey Penney, Nicholas C Cronin, Owen Garcia-Perez, Isabel Molloy, Michael G Holmes, Elaine Shanahan, Fergus Cotter, Paul D O'Sullivan, Orla</td>
<td>2018</td>
<td>Human</td>
<td>Metabolic phenotyping and metagenomic analysis of the gut microbiome of 40 professional international rugby union players. Whole metagenome shotgun sequencing, metabolic phenotype of the players and the gut microbiota.</td>
<td>Diversity of microbiota increased in professional athletes compared with high BMI and low BMI controls. Increased Akkermansia in the gut of the athlete population. Difference of 98 metabolic pathways between athlete and control cohorts. The athlete group had the highest mean difference in metabolic pathways. The SCFA levels in faecal matter showed significantly higher levels of acetate relative to control samples.</td>
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<td>Measurement of gluconeogenesis in rats fed propionate, butyrate and fructo-oligosaccharides. Model of Caco-2 cells for intestinal enterocytes, exposed to butyrate to measure G6PC and PCK1 expression as well as intracellular cAMP concentration. Assessment of whether propionate mediates intestinal gluconeogenesis through gut-brain communication through portal nervous deafferentation. Rat models were fed SCFA and fructo-oligosaccharide diets for 2 weeks. Immunofluorescence was conducted to identify FFAR3 expression in the portal vein. Exposure to beta hydroxybutyrate, an antagonist for GPR41 to identify whether this affected propionate-induced GPR41 activation.</td>
<td>Propionate and butyrate activate intestinal gluconeogenesis. Propionate-induced gluconeogenesis accounted for 23% of total glucose production. In vitro exposure to butyrate resulted in G6PC and PCK1 expression as well as a 3 fold increase of intracellular cAMP. Propionate induces IGN gene expression via a gut-brain neural circuit. Propionate could not increase G6Pase activity and PCK1, but butyrate could. Propionate activation can be reversed by Beta-hydroxybutyrate. Dietary propionate causes a 2 to 3-fold increase in the expression of c-Fos. Neuronal activation was not seen in capsaicin- treated rats.</td>
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<td>The Gut Microbiota Suppresses Insulin-Mediated Fat Accumulation Via The Short-Chain Fatty Acid Receptor GPR43.</td>
<td>Kimura, Ikao Ozawa, Kentaro Inoue, Daishuke Imamura, Takeshi Kimura, Kumi Maeda, Takeshi Terasawa, Kazuya Kashihara, Daiji Hirano, Kanako Tani, Taeko Takahashi, Tomoyuki Miyachi, Satoshi Shioi, Go Inoue, Hiroshi Tsujimoto, Gozoh</td>
<td>2013</td>
<td>Mouse</td>
<td>P2 promoter-driven adipose-specific Gpr43 transgenic mice. Exposure with acetate followed by bolus of insulin was administered intraperitoneally. Measurement of Akt phosphorylation of Ser473. Evaluation of SCFA and GPR43 activity on insulin signalling in white adipose tissue. Differences in acute-suppressed insulin impact were seen in distinct mouse and tissue. Lipoprotein lipase activity was higher in GPR43 knockout mice and lower in the Ap2-promoter driven transgenic mice. Acetate suppressed insulin-induced glucose and fatty acid uptake in adipocytes, not in Gpr43 knockout mice. Plasma triglycerides were low in Gpr43 knockout mice fed high fat diets when compared with aP2-GPR43 transgenic mouse. This effect was not demonstrated in germ-free mice or after antibiotics, indicating a role of the gut microbiota. GPR43 promotes energy expenditure through uptake of glucose and lipids into other tissues other than adipose.</td>
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<td><strong>ANGPTL4 Expression Induced by Butyrate And Rosiglitazone In Human Intestinal Epithelial Cells Utilizes Independent Pathways.</strong></td>
<td>Korecka, Agata de Wouters, Tomas Cultrone, Antonietta Lapuque, Nicolas Petersson, Sven Doré, Joël Blottière, Hervé M Arulampalam, Veilmurugesan</td>
<td>2013</td>
<td>Cell Model + mouse model</td>
<td>Using cell lines, exposed to butyrate or rosiglitazone. This was repeated in HT-29 cells - as this showed largest initial response. Butyrate administration to germ-free mice, comparison with sterile water as a control. Measured ANGPTL4 mRNA expression in epithelial cells. Measured PPAR-γ in HT-29 cells treated with butyrate acetate and propionate. Introduction of <em>C. tyrobutyricicum</em> to germ-free mice. Measured induction of ANGPTL4 expression in ileal and colonic epithelium samples. All 3 cell lines showed an increase in ANGPTL4 transcription when exposed to butyrate. When treating the cells with butyrate, the level of PPAR-γ was lower. Butyrate-induced ANGPTL4 expression is not dependent on PPAR-γ signalling.</td>
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<td><strong>Microbial Modulation Of Energy Availability In The Colon Regulates Intestinal Transit.</strong></td>
<td>Wichmann, Anita Allibhary, Ava Greiner, Thomas U Pkvier, Hubert Lundén, Gunnel Ostergren Larsson, Thomas Drucker, Daniel J Deleenheer, Nathalie M Cani, Patrice D Bäckhed, Fredrik</td>
<td>2013</td>
<td>Mice</td>
<td>The authors compared portal vein expression of GLP-1 in germ-free mice to wild type mice. Transplanted microbiota from a wild type mouse and measured Gcg expression in the colon after 24 and 72 hr. Incubation of proximal colon tissue with either a physiological concentration of SCFAs or saline as a control. The absence of microbially-produced SCFAs in germ-free mice colon results in significantly higher plasma GLP-1 levels compared with germ free control mice. After 72 hours the number of GLP-1 expressing cells decreased to baseline levels. Colonic tissue when exposed to SCFAs showed reduction in Gcg expression in GF colon.</td>
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<td>Activation Of Short And Long Chain Fatty Acid Sensing Machinery In The Ileum Lowers Glucose Production In Vivo.</td>
<td>Zadeh-Tahmasebi, Melika Duca, Frank A Rasmussen, Brittany A Bauer, Paige V Côté, Clémence D Filippi, Beatrice M Lam, Tony K T</td>
<td>2016</td>
<td>Rat</td>
<td>Propionate infusion into ileum whilst using pancreatic clamping to maintain plasma glucose measurements. They used viral knockdown of FFAR2. Then compared to GLP-1 antagonist and repeated experiment. Additionally authors used local anaesthetic infusion to limit influence of gut vagal afferents.</td>
<td>In wild type mice, propionate infusion for 50 minutes resulted in an increase in glucose needed to maintain euglycemia. Ileal infusion with propionate resulted in an increase glucose demand to maintain euglycemia. These effects were not seen with plasma infusion. Propionate sensing is dependent on the activation of FFAR2.</td>
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