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The relationship between BMI and metabolomic profiles: a focus on amino acids

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The role of metabolomics in the field of nutrition is continuing to grow and it has the potential to assist in the understanding of metabolic regulation and explain how minor perturbations can have a multitude of biochemical endpoints. It is this development, which creates the potential to provide the knowledge necessary to facilitate a more targeted approach to nutrition. In recent years, there has been interest in applying metabolomics to examine alterations in the metabolic profile according to weight gain/obesity. Emerging from these studies is the strong evidence that alterations in the amino acid (AA) profiles are associated with obesity. Several other studies have also shown a relationship between branched-chain amino acids (BCAA), obesity and insulin resistance. The present review focuses on the proposed link between AA and in particular BCAA, obesity and insulin resistance. In conclusion, a wealth of information is accumulating to support the role of AA, and in particular of the BCAA, in obesity.

Metabolomics: Amino acids: BMI: Branched-chain amino acids

Obesity is now considered as a major global health problem, and the WHO has demonstrated that obesity levels have reached epidemic proportions worldwide with approximately 2.3 billion adults predicted to be overweight or obese by the year 2015⁽¹⁾. It is well recognised that obesity plays a central role in insulin resistance, the metabolic syndrome and type-2 diabetes mellitus. Despite many years of research the exact mechanisms underlying the role of obesity in the development of these disorders and diseases are still not fully elucidated. However, in recent years, the application of 'omic' technologies to studies comparing lean and obese subjects has enhanced our understanding of this research area. This review will focus on the literature, which has utilised metabolomic techniques to investigate the altered metabolic profile in obesity and the subsequent effect on insulin resistance.

Metabolomics

Metabolomics is the comprehensive study of metabolites in biofluids, tissues or cellular extracts. The metabolic profile of a sample may be assessed using a variety of techniques including Proton NMR spectroscopy, LC–MS and GC–MS. The role of metabolomics in the field of nutrition is continuing to grow and its utility in a number of studies has been demonstrated^(2,3).

Earlier applications of metabolomics in this field compared metabolic profiles of lean and obese subjects (Table 1). Pietiläinen *et al.*⁽⁴⁾ investigated whether acquired obesity was associated with changes in global serum lipid profiles independent of genetic factors in young adult monozygotic twins. In this study, fourteen healthy monozygotic pairs discordant for obesity (10–25 kg weight

Abbreviations: AA, amino acid; BCAA, branched-chain amino acid; GBP, gastric bypass surgery; HF, high fat.

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Table 1. Overview of metabolomics based studies investigating alterations related to BMI

Reference	Target population (n)	Methodology	Outcome
Oberbach <i>et al.</i> ⁽⁶⁾	Lean group: BMI 23–25 kg/m ² (n 15) Obese group: BMI 30–45 kg/m ² (n 15)	Serum samples; targeted metabolomics	Twelve metabolites including amino acids and fatty acids were identified as significantly related to obesity
Newgard <i>et al.</i> ⁽⁷⁾	Lean group: median BMI 36.6 kg/m ² (n 67) Obese group: median BMI 23.2 kg/m ² (n 74)	Serum samples; targeted metabolomics	A distinctive metabolic ‘signature’ related to BCAA catabolism emerged in the obese subjects which was related to HOMA-IR Score
Pietilainen <i>et al.</i> ⁽⁴⁾	Monozygotic twin pairs discordant for obesity (n 14) Lean group: BMI 24.5–26.1 kg/m ² Obese group: BMI 28.4–32.5 kg/m ² Weight concordant control pairs (n 10) Lean group: BMI 20.8–23.7 kg/m ² Obese group: BMI 26.9–30.4 kg/m ²	Serum samples; untargeted metabolomics	Obesity without interaction of genetics, was associated with alterations in the lipid metabolism
Kochhar <i>et al.</i> ⁽⁵⁾	Low BMI group: BMI < 21 kg/m ² (n 96) High BMI group: BMI > 25 kg/m ² (n 54)	Plasma and urine samples; untargeted metabolomics	Differences both in the AA and lipid profiles between low and higher BMI metabolomic profiles
Huffman <i>et al.</i> ⁽⁹⁾	Overweight/obese group: BMI 25–35 kg/m ² (n 73)	Plasma samples; targeted metabolomics	Neutral AA and fatty acids were independently associated with insulin resistance
Tai <i>et al.</i> ⁽¹⁰⁾	Non-obese group: median BMI 24 kg/m ² (n 263)	Plasma samples; targeted metabolomics; Urine samples; targeted metabolomics	Insulin resistance was correlated with increased levels of a cluster of BCAA and related AA
Shaham <i>et al.</i> ⁽¹¹⁾	Healthy group 1: BMI 18.3–26.9 kg/m ² (n 22) Healthy group 2: BMI 19.0–31.5 kg/m ² (n 25) Pre-diabetics group: BMI 18.8–41.2 kg/m ² (n 25)	Serum samples; targeted metabolomics	BCAA were among a cluster of metabolites that changed significantly in insulin resistant v. normal participants
Wang <i>et al.</i> ⁽¹²⁾	Normoglycemic individuals of which 201 developed diabetes: BMI 22.5–30.55 kg/m ² (n 189 for metabolomics analysis)	Plasma samples; targeted metabolomics	Five BCAA and aromatic AA had highly significant associations with future diabetes: a combination of three AA predicted future diabetes with a more than fivefold higher risk for individuals in top quartile
Laferarre <i>et al.</i> ⁽¹⁴⁾	Gastric bypass patients: BMI > 35 kg/m ² (n 10) Dietary weight loss patients: BMI > 35 kg/m ² (n 11)	Plasma samples; targeted metabolomics	Total AA, BCAA and their derivatives decreased after GBP, but not after dietary intervention

AA, amino acid; BCAA, branched-chain amino acid; GBP, gastric bypass surgery; HOMA, The Homeostatic Model Assessment.

difference) and ten weight concordant control pairs aged 24–27 years were identified from a large population-based study. Insulin sensitivity was assessed and body composition was measured by dual-energy X-ray absorptiometry (percentage of body fat) and by MRI (subcutaneous and intra-abdominal fat). Non-targeted analysis of the lipid profiles revealed that obesity, independent of genetic influences was primarily related to an increase in lysophosphatidylcholines and decrease in diethyl ether phospholipids, and these changes were associated with insulin resistance. Kochhar *et al.*⁽⁵⁾ employed a slightly different approach by comparing individuals with varying BMI. In this study, multivariate analysis of Proton NMR spectra of urine and plasma from 150 healthy human subjects were acquired and distinct separation in the metabolomic profiles between low-BMI cohort (BMI < 21 kg/m²) and high-BMI cohort (BMI > 25 kg/m²) was observed. Further analysis revealed significant differences both in the amino acid (AA) and lipid profiles according to BMI. Based on the metabolomic differences the hypothesis emerged that in young people and/or individuals with low BMI, females had higher rates of lipid biosynthesis compared with males, whereas males had higher rates of protein turnover than females.

In a more recent study, Oberbach *et al.*⁽⁶⁾ combined proteomic and metabolomic approaches to identify circulating molecules that discriminated fifteen healthy lean from fifteen healthy obese individuals (lean group: BMI 23–25 kg/m² and obese group: BMI 30–45 kg/m²). To correct the variations in physical activity, the subjects performed a 1 h exercise bout to exhaustion. Targeted metabolomic analysis was carried out on serum samples: out of the 1683 metabolites analysed, twelve significantly related to obesity. Obese individuals had raised levels of glycine, glutamine and glycerophosphatidylcholine 42:0 and decreased levels of diacylglycerophosphatidylcholines (PCaa) (PCaa 32:0, PCaa 32:1 and PCaa 40:5).

Similar to the Oberbach study, Newgard *et al.*⁽⁷⁾ discovered differences in the AA profiles of obese individuals compared with lean individuals. In this study, comprehensive metabolic profiling was performed on seventy-four obese (median BMI of 36.6 kg/m²) and sixty-seven lean (median BMI of 23.2 kg/m²) subjects. ‘Healthy’ obese subjects, free of diabetes or other serious illness were selected; however, not surprisingly, they found the obese participants to be more insulin resistant, on average, compared with lean controls. Through metabolomic analysis a distinctive metabolic ‘signature’ related to branched-chain amino acid (BCAA) catabolism emerged in the obese subjects. This metabolic signature comprised a combination of BCAA (leucine/isoleucine and valine), methionine, glutamate/glutamine, the aromatic AA phenylalanine and tyrosine and C₃ and C₅ acylcarnitines. In addition, there was a significant linear relationship between this BCAA-related metabolite component and The Homeostatic Model Assessment-IR Score. To further investigate these findings an animal study was performed: supplementation of a high-fat (HF) diet with BCAA (HF/BCAA) reduced food intake and body weight, but caused the animals to be equally insulin resistant compared with heavier animals fed on a non-supplemented HF diet. The

HF/BCAA-induced insulin resistance was accompanied by chronic phosphorylation of the mammalian target of rapamycin, c-Jun N-terminal kinase and insulin receptor substrate 1 and was reversed by the mammalian target of rapamycin inhibitor, rapamycin. Stemming from this work, a hypothesis emerged that proposed that deregulation of BCAA metabolism in a background of HF consumption makes an independent contribution to the development of obesity-associated insulin resistance.

This initial study has been followed up in a number of different study cohorts (Table 1) including subjects with type-2 diabetes mellitus. Comparison of metabolomic profiles in fasted obese type-2 diabetic subjects against obese non-diabetic subjects revealed increased circulating concentration of AA including leucine and valine in the type-2 diabetic subjects⁽⁸⁾. In a study of individuals at risk of type-2 diabetes mellitus⁽⁹⁾, neutral AA, including the BCAA valine and leucine/isoleucine were independently associated with insulin resistance. Similarly, in a study of Asian and Chinese men⁽¹⁰⁾, 263 non-obese (BMI approximately 24 kg/m²) subjects were selected for MS-based metabolic profiling of acylcarnitines, AA and organic acids combined with hormonal and cytokine profiling. After controlling for BMI, insulin resistance was correlated with increased levels of alanine, proline, valine, leucine/isoleucine, phenylalanine, tyrosine, glutamate/glutamine and ornithine. Overall, the findings from this study demonstrate that perturbations in AA homeostasis, including BCAA were associated with insulin resistance in individuals with relatively low body mass but not inflammatory markers. One of the first studies to analyse the metabolomic response to an oral glucose challenge test⁽¹¹⁾ reported that BCAA were among a cluster of metabolites that changed significantly in insulin resistant *v.* normal participants. Moreover, leucine/isoleucine and glycerol were jointly predictive of fasting insulin levels.

The predictive ability of AA concentration including BCAA was revealed in a recent study. Wang *et al.*⁽¹²⁾ demonstrated that fasting plasma concentrations of BCAA, phenylalanine and tyrosine were elevated up to 12 years prior to the onset of diabetes in high-risk subjects. In this study 2422 normoglycemic individuals were followed for 12 years and 201 developed diabetes. AA, amines and other polar metabolites were profiled in baseline samples by LC-MS/MS in 189 cases who developed diabetes and in 189 matched controls. Targeted metabolomic analysis revealed that five AA including BCAA and aromatic AA had highly significant associations with future diabetes. A combination of three of the AA predicted future diabetes with a more than 5-fold higher risk for individuals in the top quartile of amino concentrations. The results were then replicated and validated in an independent, prospective cohort: subjects (163 cases and 163 controls) were selected from the Malmö Diet and Cancer study (mean age 58 years, 55% women) and the five AA of interest were measured. Four of the five individual AA (leucine, valine, tyrosine and phenylalanine) were significantly associated with incidence of diabetes. Furthermore, a three-AA combination (isoleucine, phenylalanine and tyrosine) was examined revealing subjects in the upper quartile of the three-AA combination to have a 4-fold higher risk of

diabetes compared with those in the lowest quartile. Overall, their findings emphasise the potential key role of AA metabolism early in the pathogenesis of diabetes and suggest that AA profiles could aid in diabetes risk assessment.

Further evidence has recently emerged to support the importance of BCAA in insulin resistance through the study of weight loss and gastric bypass surgery (GBP). Five hundred participants (average BMI 33.91 (SD 4.7) kg/m²) were recruited from a larger study population (WLM clinical trial), and targeted metabolomic analysis revealed a biosignature reporting on BCAA metabolism that was associated with insulin resistance in non-diabetic overweight/obese individuals. Furthermore, this metabolic signature predicted improvement in insulin resistance with moderate weight loss independent of the amount of weight lost⁽¹³⁾.

In a separate study of subjects undergoing GBP further evidence emerged to support the important role of BCAA in insulin resistance⁽¹⁴⁾. It is well established that glycemic control improves more after GBP compared with equivalent diet-induced weight loss in patients with morbid obesity and type-2 diabetes mellitus. However, the mechanisms of this better metabolic response after GBP are relatively unknown. With this in mind a metabolomic study was performed on ten subjects undergoing GBP and eleven subjects undergoing a dietary intervention weight loss programme. AA and acylcarnitines were measured prior to and following a matched 10 kg weight loss induced by GBP or diet. Analysis revealed that the total AA, BCAA and their derivatives decreased after GBP, but not following the dietary intervention. The authors conclude from this study that the enhanced decrease in circulating AA after GBP occurs by mechanisms other than weight loss and may contribute to better improvement in glucose homeostasis observed with surgical intervention.

In summary, the earlier studies demonstrate that there are significant relationships between BCAA and insulin resistance and importantly their relationships were demonstrated in studies that included obese and lean subjects. However, it remains to be proven whether the BCAA are mediators of insulin resistance or by-products of the associated metabolic dysfunction. To address this, a number of animal studies have been performed. Supplementation of an HF diet with all three BCAA promoted the development of insulin resistance in rats⁽⁷⁾, whereas an infusion of a mixture of eighteen AA in human subjects also resulted in reduced insulin sensitivity⁽¹⁵⁾. However, all the evidence is not so straightforward and some studies have demonstrated positive effects on glucose tolerance and also on body weight following supplementation with the BCAA leucine^(16–25), whereas others report no effects⁽²⁶⁾. Further mechanistic work is needed in this field to provide a clear understanding of the molecular mechanisms underpinning altered BCAA metabolism.

Although diet plays an influential role, other factors can contribute to the maintenance of raised levels of BCAA in obese and insulin resistant individuals. Some of the possible mechanisms that have been suggested include reduced activities of key BCAA catabolic enzymes in liver and adipose tissue^(27,28), and differential rates of protein

turnover. However, more evidence is required in order to fully understand changes in flux through metabolic pathways that may lead to increased levels of BCAA and related metabolites in insulin resistant individuals. Furthermore, to date, no clear mechanism has emerged to explain the role of BCAA in insulin resistance and obesity and further research will be required to delineate the exact role of BCAA in the development of insulin resistance. Despite this, the earlier reviewed studies present compelling evidence for an altered BCAA signature as a 'biomarker signature' for diabetes risk. However, it is important to realise that, although a focus on BCAA has emerged in the literature, alterations in other AA such as phenylalanine, tyrosine and sulphur AA have been consistently reported^(12,29). The true predictive biomarker potential may lie in the use of the BCAA in conjunction with some of these other AA.

Conclusion

In conclusion, there is strong evidence to support the hypothesis that BCAA and related metabolites are associated with insulin resistance and diabetes. However, the molecular mechanism leading to the increased levels of circulating BCAA remains to be identified. Progress in this field is required to enhance our understanding of the role of BCAA in the development of insulin resistance and to support their use as markers of future diabetes risk.

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