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RESEARCH ARTICLE

Gene expression response of mouse lung, liver and white adipose tissue to β-carotene supplementation, knockout of *Bcmo1* and sex

Yvonne G. J. van Helden[1,2], Roger W. L. Godschalk[2], Johannes von Lintig[3], Georg Lietz[4], Jean-Francois Landrier[5], M. Luisa Bonet[6], Frederik J. van Schooten[2] and Jaap Keijer[1]

[1] Human and Animal Physiology, Animal Sciences, Wageningen University, Wageningen, The Netherlands
[2] Department of Health Risk Analysis and Toxicology, Research Institute NUTRIM, Maastricht University, Maastricht, The Netherlands
[3] Department of Pharmacology, School of Medicine, Case Western Reserve University, OH, USA
[4] School of AFRD, Newcastle University, UK
[5] UMR 1260 INRA/Universite Aix-Marseille I et II, Marseille, France
[6] Laboratory of Molecular Biology, Nutrition and Biotechnology, University of the Balearic Islands (UIB) and CIBER de Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Palma de Mallorca, Spain

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Scope: Little information is available on differences, commonalities and especially interactions in overall gene expression responses as a result of diet, differences in sex (male and female) and effects induced by differences in metabolism. Moreover, it is unknown whether such effects are tissue specific.

Methods and results: We investigated the gene expression effects induced by β-carotene (BC) supplementation, knockout of *β-carotene 15,15′-monooxygenase 1* (*Bcmo1*) and differences between male and female mice in lung, liver and inguinal white adipose tissue (iWAT). Unsupervised principal component analysis showed that lung gene expression was most affected by knockout of *Bcmo1*. Liver was most affected by knockout of *Bcmo1* and differences in sex. iWAT was most affected by differences in sex. Hardly any genes were commonly influenced by BC among the three tissues. The effect of BC supplementation and knockout of *Bcmo1* were relatively sex specific, especially in iWAT.

Conclusion: These data demonstrate that gene expression differences induced by BC are limited to the tissue and sex that is analyzed, and that differences in metabolism induced by for example single nucleotide polymorphisms, should be taken into account as much as possible. Moreover, our results indicate that translation from one tissue to the other should be done with caution for any nutritional intervention.

Keywords:
Gender differences / Metabolism / Tissue specificity / Vitamin A / Whole genome microarray analysis

1 Introduction

To test specific hypotheses in nutritional research, mechanistic studies are often performed in rodents and nutritional effects are usually assessed within one specified genetic background, tissue and sex. For this reason, little information is available on commonalities and differences in response to dietary interventions between different tissues, genetic backgrounds and a difference in sex. This type of information is necessary to interpret results and to generalize and extrapolate effects between tissues. This is for example of importance for benefit–risk assessment, since

Correspondence: Dr. Jaap Keijer, Human and Animal Physiology, Animal Sciences, Wageningen University, P. O. Box 338, 6700 AH Wageningen, The Netherlands
E-mail: jaap.keijer@wur.nl
Fax: +31-317-484077

Abbreviations: BC, β-carotene; ***Bcmo1,*** *β-carotene15,15′-monooxygenase1*; ***Bcmo1*$^{-/-}$,** *Bcmo1* knockout mouse; ***Bcmo1*$^{+/+}$,** wild-type (mice); **iWAT,** inguinal white adipose tissue; **Co,** control; **PBMC,** peripheral blood mononuclear cell; **PCA,** principal component analysis

risks and benefits of a compound should be based on all tissues. Moreover, surrogate tissues are increasingly used in mechanistic studies in humans [1]. Surrogate tissues are tissues, such as peripheral blood mononuclear cells (PBMCs), that are relatively well accessible in humans and are used to explore a research question and to extrapolate those effects to other tissues. However, it is unknown whether results obtained from surrogate tissues can indeed be extrapolated with confidence to a target tissue. A comparison between gene expression in different tissues will give insight into the organ specificity of changes induced by an intervention. We will address three important questions in nutritional research in this paper; ''how many genes are changed by a change in nutritional intake compared to the number of genes changed by a difference in metabolism or sex and do they interact'', ''do different tissues differ in response to an nutritional intervention?'' and finally ''do female and male mice respond similar to a dietary intervention''? We used a previously performed study to analyze these questions. In this study we investigated the effects of β-carotene (BC) in male or female mice with a knockout or not for *β-carotene 15,15′-monooxygenase 1* (*Bcmo1*), a key enzyme in BC metabolism, on gene expression changes in lung, liver and inguinal white adipose tissue (iWAT). An in-depth analysis of the effect of BC in the lung of *Bcmo1* knockout mice ($Bcmo1^{-/-}$) was published previously [2–5].

BC is a dietary compound that is present in colored fruits and dark green vegetables and is frequently used as a food-coloring agent. After absorption and metabolism in the intestine, remainder intact BC is transported to the liver where BC is metabolized and, like in white adipose tissues, stored [6, 7]. Increased BC intake has been associated with many health-promoting activities, including antioxidant properties to prevent radical-induced macromolecular damage [8]. It is associated with a decreased risk for cardiovascular diseases [9] and a decreased risk for several types of cancer [10]. Finally, BC and its metabolites such as vitamin A are functionally active in many different organs, having a major role in, for example, organism development, sperm production and vision [11–13]. However, besides these important functional and health-promoting effects, a high intake of BC has been associated with an increased lung cancer risk in smokers and asbestos-exposed subjects [2, 14]. Moreover, the increased lung cancer risk was higher in females than in males and was more persistent in female volunteers than in male volunteers after the intervention [15].

An important drawback in BC research is the lack of an appropriate animal model that resembles human BC metabolism and at the same time allows the application of state-of-the-art genetic and functional genomic knowledge and tools, such as commercial whole genome microarrays. Compared with humans, rodents differ in the activity of Bcmo1. Bcmo1 symmetrically cleaves BC to produce two retinal molecules [16, 17], which can be further metabolized into several other downstream metabolites [18]. A *Bcmo1* knockout mouse ($Bcmo1^{-/-}$) model has become available. BC supplementation to these mice results in a higher degree of BC accumulation in several tissues [19]. Therefore, this model makes it possible to study specifically the effects of BC, and its dependency on the BC-metabolizing enzyme Bcmo1. As a result, this model can provide more insight into BC biology.

In this manuscript, we aimed to provide insight into the gene expression changes induced by BC supplementation, knockout of $Bcmo1^{-/-}$ or sex in lung, liver and iWAT. Therefore, we used whole genome microarray analyses to assess the number of genes differentially expressed in lung, liver and iWAT of male and female $Bcmo1^{-/-}$ and wild-type ($Bcmo1^{+/+}$) mice given a diet with or without BC (150 mg/kg diet) for 14 wk to contribute to questions of general importance to nutritional research as well as to BC research specifically.

2 Materials and methods

2.1 Animals and treatment

Twelve female and 12 male B6129SF1 ($Bcmo1^{+/+}$) and 12 female and 12 male B6;129S-$Bcmo1^{tm1dnp}$ ($Bcmo1^{-/-}$) mice [19] were used for the experiment. The mouse experiment was conducted in accordance with the German animal protection laws by the guidelines of the local veterinary authorities. During the breeding and weaning periods of the mice, mothers were maintained on KLIBA 3430 chow containing 14 000 IU vitamin A/kg diet (Provima Kliba AG, Kaiseraugst, Switzerland). Five-wk-old female and male $Bcmo1^{+/+}$ and $Bcmo1^{-/-}$ mice were caged in groups containing two to four siblings per group and were maintained under environmentally controlled conditions (temperature 24°C, 12 h/12 h light/dark cycle). Mice had ad libitum access to feed and water. Basic feed consisted of the palletized diet D12450B (Research Diets, USA) with a fat content of 10% and a defined fatty acid composition. The diet was modified to contain 1500 IU vitamin A/kg diet, which is a vitamin A-sufficient diet, and the control diet (control) was supplemented with water-soluble vehicle beadlets (DSM Nutritional Products, Basel, Switzerland) containing DL-α-tocopherol and ascorbyl palmitate as stabilizers, as well as carriers such as gelatine, corn oil sucrose and starch. The BC diet (BC) was supplemented with identical water-soluble beadlets containing BC (DSM Nutritional Products) to generate 150 mg BC/kg diet. Beadlets were added by the manufacturer before low temperature pelletting. Feed pellets were color marked and stored at 4°C in dark.

After 14 wk of dietary intervention, six female and six male $Bcmo1^{+/+}$ mice on the control diet ($Bcmo1^{+/+}$ Co), six female and six male $Bcmo1^{+/+}$ mice on the BC diet ($Bcmo1^{+/+}$ BC), three female and six male $Bcmo1^{-/-}$ mice on the control diet ($Bcmo1^{-/-}$ Co) and three female and six male $Bcmo1^{-/-}$

mice on the BC diet (*Bcmo1*$^{-/-}$ BC) were randomly sacrificed during three subsequent mornings. Blood was collected from the vena cava after isoflurane and ketamin anethesia. Blood was coagulated for at least 20 min at room temperature, cooled to 4°C and centrifuged. Lung, iWATs and liver tissues were removed, lung and liver tissue were rinsed in PBS and all tissues were snap frozen in liquid nitrogen. The tissues were stored at −80°C until use. Due to an insufficient number of female *Bcmo1*$^{-/-}$ mice in the original breeding pool, female *Bcmo1*$^{-/-}$ mice were used that were born 2 wk later from an identical experiment, treated identically with three mice on the control diet and three mice on the BC diet to generate $n = 6$ per group, and sacrificed 2 wk after the first group of mice.

2.2 RNA isolation

Lung, iWAT and liver were homogenized in liquid nitrogen using a cooled mortar and pestle. Total RNA was isolated using TRIzol reagent (Invitrogen, Breda, The Netherlands) followed by purification using RNeasy columns (Qiagen, Venlo, The Netherlands) using the instructions of the manufacturer. RNA concentration and purity were measured using the Nanodrop system (IsoGen Life Science, Maarsen, The Netherlands). RNA degradation was checked on the Experion (Bio-Rad, Veenendaal, The Netherlands) using Experion StdSense chips (Bio-Rad). Three RNA samples did not meet RNA quality (RNA from lung from one individual female *Bcmo1*$^{+/+}$ control diet mouse, RNA from lung from one individual female *Bcmo1*$^{+/+}$ BC diet mouse and RNA from iWAT from one individual male *Bcmo1*$^{+/+}$ BC diet mouse), which were omitted from the experiment. Knockout of *Bcmo1* was confirmed using quantitative RT-PCR analysis (unpublished results) and for several genes expression was checked using real-time quantitative Q-PCR and was found to be identical to the microarray results [2–5].

2.3 Microarray hybridization procedure

The 4 × 44k Agilent whole mouse genome microarrays (G4122F, Agilent Technologies, Santa Clara, CA, USA) were used. Preparation of the sample and the microarray hybridization were carried out according to the manufacturer's protocol with a few exceptions as described previously [20, 21]. In brief, cDNA was synthesized from 1 μg RNA from individual samples using the Agilent Low RNA Input Fluorescent Linear Amplification Kit for each animal without addition of spikes. Thereafter, samples were split in two equal amounts, to synthesize Cyanine 3-CTP (Cy3) and Cyanine 5-CTP (Cy5) labeled cRNA using half the amounts per dye as indicated by the manufacturer (Agilent Technologies). Labeled cRNA was purified using RNeasy columns (Qiagen). Yield, A_{260}/A_{280} ratio and Cy3 or Cy5 activity were examined for every sample using the nanodrop. All samples met the criteria of a cRNA yield higher than 825 ng and a specific activity of at least 8.0 pmol Cy3 or Cy5. Aliquots of 1200 ng of every Cy3-labeled cRNA sample were pooled and used as a common reference pool. Individual 825 ng Cy5-labeled cRNA and 825 ng pooled Cy3-labeled cRNA were fragmented in 1 × fragmentation and 1 × blocking agent (Agilent Technologies) at 60°C for 30 min and thereafter mixed with GEx Hybridization Buffer HI-RPM (Agilent Technologies) and hybridized in a 1:1 ratio at 65°C for 17 h in an Agilent Microarray Hybridization Chamber rotating at 4 rpm. After hybridization, slides were washed according to the wash protocol with stabilization and drying solution (Agilent Technologies). Arrays were scanned with an Agilent scanner with 10 and 100% laser power intensities (Agilent Technologies).

2.4 Data analyses of microarray results

Signal intensities for each spot were quantified using Feature Extraction 9.1 (Agilent Technologies). Median density values and background values of each spot were extracted for both the experimental samples (Cy5) and the reference samples (Cy3). Quality control for every microarray was performed visually, by using quality control graphs from Feature extraction and M-A plots and boxplots that were made using limmaGUI in R (Bioconductor) [22]. Data were imported into GeneMaths XT 2.0 (Applied Maths, Sint-Martens-Latem, Belgium). Spots with a Cy5 and Cy3 signal twice above background were selected and log transformed. The Cy5 signal was normalized against the Cy3 intensity as described before [23]. Principal component analysis [2] was performed using GeneMaths XT.

2.5 Statistical analysis

General effects of diet, sex and genotype on concentrations of BC, retinol and retinyl esters in lung, liver and iWAT were analyzed using 2 × 2 × 2 factorial univariate ANOVA (SPSS version 15.0) and considered statistically significant when $p < 0.05$. Fold changes for microarray gene expression were calculated using mean log signal intensities. *p*-Values for differential expressions were calculated between two groups using two-tailed Student's *t*-test statistics on log intensity values. Changes were considered statistically significant at $p < 0.05$.

3 Results

3.1 Responses of BC supplementation related to *Bcmo1* knockout and sex in lung, liver and iWAT

BC accumulation was highest after BC supplementation in *Bcmo1*$^{-/-}$ mice and reached similar levels in iWAT and

liver, while accumulation was lower in the lung. Depending on the experimental group, retinol concentrations were between five- and tenfold higher in the liver compared with iWAT and lung tissue (Fig. 1).

Whole genome gene expression changes were analyzed using microarray technology. Spots with a signal twice above background were regarded as positive spots and included in the analysis. This resulted in 31 128, 24 746 and 25 139 spots with a signal twice above background in lung, liver and iWAT, respectively. Principal component analysis (PCA) was used as an unsupervised tool to plot the individual animals based on their overall gene expression profile. In lung, the individual animals were most discriminated based on the presence or absence of intact *Bcmo1* (Fig. 2A). In liver, the largest discriminating effect was observed for sex, followed by a clear discrimination based on the presence of an active Bcmo1 enzyme. This resulted in a PCA plot displaying four separated groups (Fig. 2B). Gene expression effects in iWAT were strongest differentiated between male and female mice (Fig. 2C).

3.2 Number of genes regulated by BC, *Bcmo1* genotype and sex in lung, liver and iWAT

As can be seen in Table 1, there were large differences in the number of significantly regulated genes ($p<0.05$) due to BC supplementation (BC diet versus control diet), knockout of *Bcmo1* ($Bcmo1^{-/-}$ versus $Bcmo1^{+/+}$) or difference in sex (male versus female gene expression). These effects also depended on the tissue.

BC supplementation resulted in the highest number of significantly regulated genes in iWAT of female $Bcmo1^{+/+}$ mice. These 4970 significantly regulated genes were highly dependent on sex and on the presence of Bcmo1 since BC supplementation to female $Bcmo1^{-/-}$ mice or to male

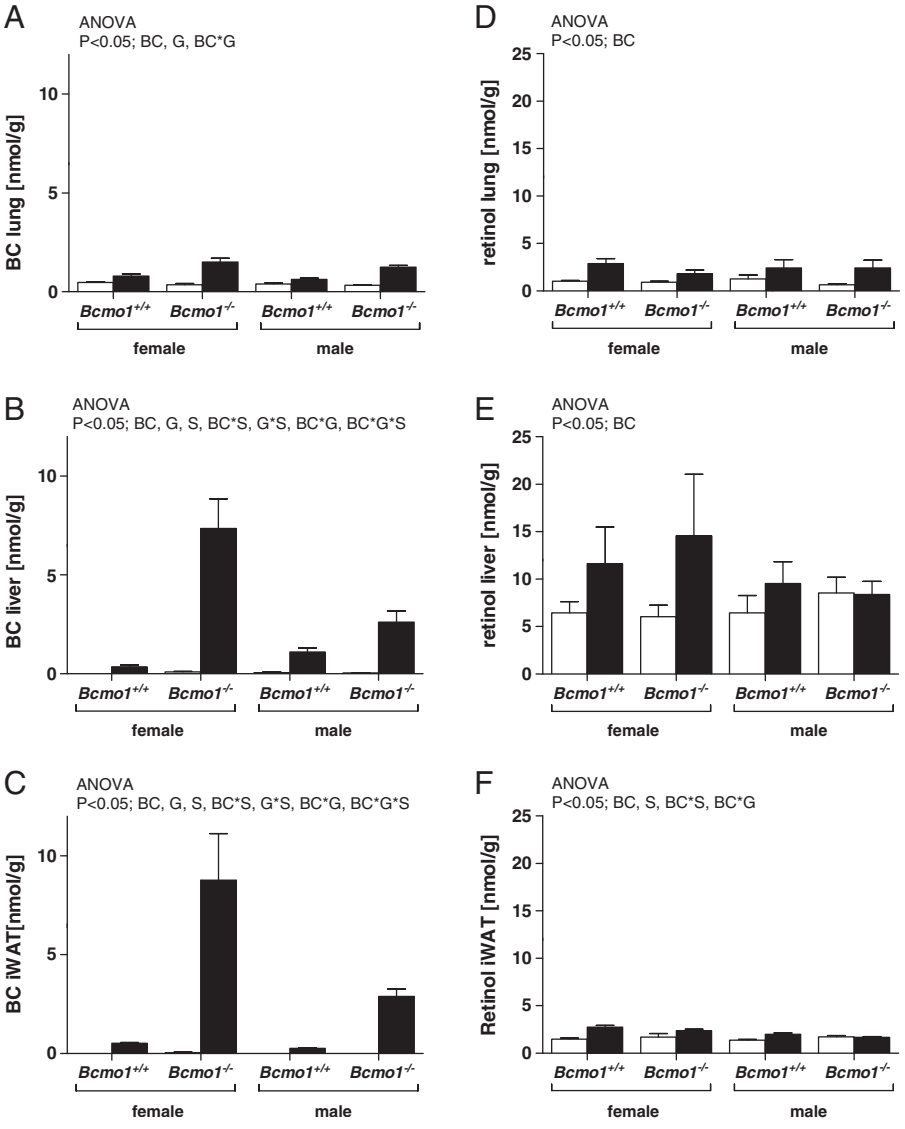

Figure 1. BC concentration in lung (A), liver (B) and iWAT (C) and concentrations of the BC metabolite retinol in lung (D), liver (E) and iWAT (F) in control mice (white bars) and in BC-supplemented mice (black bars). Data are expressed as mean ± SEM. Statistical differences were tested using ANOVA for effects of BC (BC), *Bcmo1* genotype (G) and sex (S) or any interaction, and considered statistically significant when $p<0.05$. Significant effects are displayed on top of each figure.

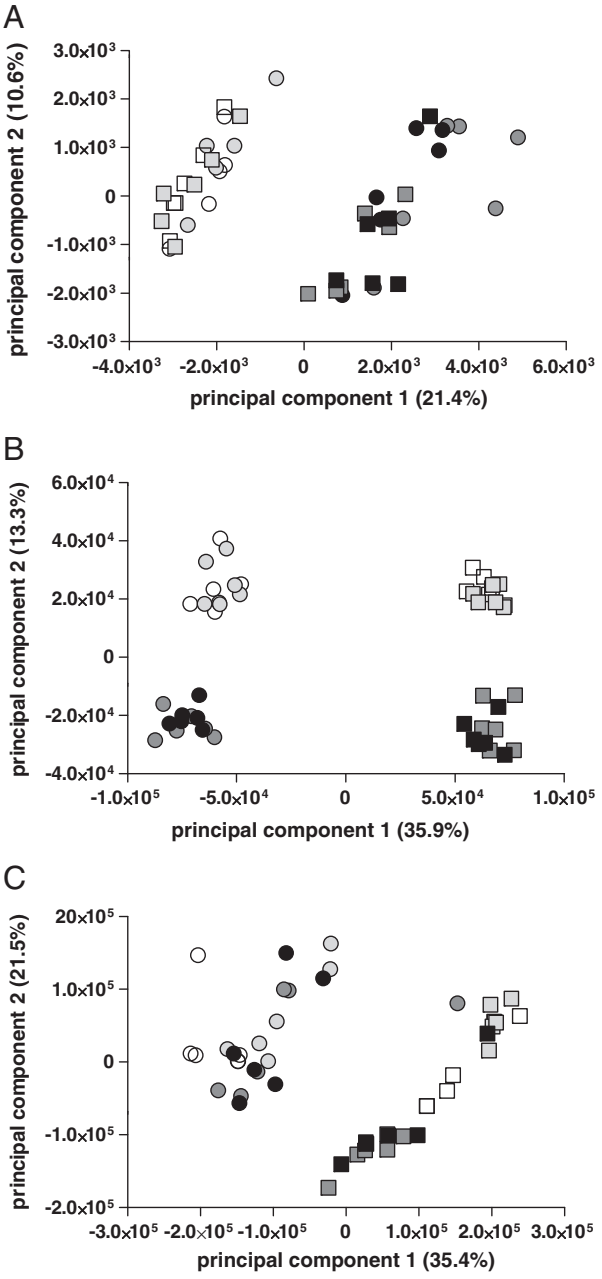

Figure 2. Unsupervised PCA of all individual animals based on differences in gene expression in lung (A), liver (B) and iWAT (C). The *x*-axis accounts for strongest variability in gene expression and the *y*-axis accounts thereafter for the strongest variability in gene expression of the data set. Individual animals are presented; *Bcmo1*$^{-/-}$ control diet (white) and *Bcmo1*$^{-/-}$ BC diet (light grey), *Bcmo1*$^{+/+}$ control diet (dark grey) and *Bcmo1*$^{+/+}$ BC diet (black) with females (squares) and males (circles).

Bcmo1$^{+/+}$ mice resulted in only 1522 and 407 significantly regulated genes, respectively. At the same stringency, BC supplementation in female *Bcmo1*$^{+/+}$ mice resulted in less (roughly ~1000) significantly regulated genes in lung and liver.

The effect of knocking out *Bcmo1,* which affects BC metabolism, was most pronounced in iWAT of male mice, resulting in 10 017 and 9546 genes significantly ($p<0.05$) differentially expressed in mice fed the control diet and mice fed the BC supplemented diet, respectively. The knockout of *Bcmo1* in iWAT of female mice resulted in 2487 and 4888 regulated genes in mice fed the control diet or mice supplemented with BC, respectively. The effect of knockout of *Bcmo1* was therefore also dependent on sex. With 2487 significantly regulated genes in iWAT of control diet fed female mice, this group had the lowest response to the presence of *Bcmo1*.

Gene expression differences between male and female were highest in iWAT tissue. There were 13 927 genes differentially expressed between male and female BC supplemented *Bcmo1*$^{+/+}$ mice. Interestingly, BC supplementation had an effect on gene expression differences in male compared to female *Bcmo1*$^{+/+}$ mice since there were "only" 8403 genes significantly differentially expressed genes between male and female *Bcmo1*$^{+/+}$ mice receiving the control diet.

3.3 Organ specificity of gene expression induced by BC, knockout of *Bcmo1* or differences in sex

We further focused on the organ specificity of significantly regulated genes induced by BC supplementation, knockout of *Bcmo1* or difference in sex. As can be seen in Table 2, gene expression differences induced by BC had the highest organ specificity, and resulted in the lowest number of common significantly regulated genes in lung, liver and iWAT. There was even not a single common gene significantly regulated by BC in male *Bcmo1*$^{-/-}$ mice in all these three tissues. Effects induced by knockout of *Bcmo1* had the lowest organ specificity, resulting in the regulation of at least 626 genes (female BC supplemented mice) in all three tissues and maximal 1261 commonly regulated genes (control diet-fed male mice). The effect of difference in sex was more tissue specific than effects induced by *Bcmo1* knockout.

4 Discussion

In this study, we investigated the effect of BC, knockout of *Bcmo1*, a key enzyme in BC metabolism, and sex on global gene expression changes in lung, liver and iWAT. We observed that BC supplementation, relative to *Bcmo1* knockout and sex, resulted in the smallest number of significantly regulated genes in any tissue and that the effect of sex was high, especially in iWAT. Moreover, the effect of BC supplementation on gene expression was highly organ specific. Altogether, our data demonstrate that effects of dietary intake should be interpreted in the context of genetic background and sex. Although in theory this may be not a surprise in vitamin A biology, the magnitude of the differences between organs and sex was unknown. In addition,

this paper shows that effects should be interpreted in relation to the investigated tissue, and warns against the use of a ''surrogate tissue'' for mechanistic research, when not properly assessed and validated.

The first question that was addressed in this study was: ''how many genes are changed by a change in nutritional intake compared to the number of genes changed by a difference in metabolism or sex and do they interact''? The number of genes changed by supplementation with the nutritional compound BC was relatively low, lower than the effects induced by knockout of *Bcmo1* and by difference in sex, which implies the need of highly powered studies to analyze effects induced by BC. The effects of BC were highly dependent on sex and metabolic activity. This implies that results should be interpreted in the context of these modifiers, which should ideally be included in any study design, especially in BC research. The lack of, for example, a metabolizing enzyme in the tissue of interest does not implicate that a change in the activity or amount of this enzyme in the whole organism might have an important effect on this specific organ. This is exemplified for the results seen for the lung. PCA showed that the knockout of *Bcmo1* was the most discriminating factor in lung. However, gene expression of *Bcmo1* was not detected at all in lung tissue in a previous study [24] and was below the background value in our microarrays. This suggests that the lung is affected indirectly, but importantly, by knockout of *Bcmo1*. This also might have implications for BC research in humans. Effects found that are induced by BC might be highly dependent on human inter-individual differences in BC conversion ability [25, 26], which are thought to occur due to polymorphisms in *Bcmo1* [27, 28].

PCA of microarray results and the number of significantly regulated genes revealed that the overall signature of gene expression patterns in all three tissues was different between genotypes. This variation might be caused either by Bcmo1 deficiency but part of this variation might also be caused by differences in the genetic background of mice. We tried to reduce the latter effect by using mixed background $Bcmo1^{+/+}$ mice and $Bcmo1^{-/-}$ mice, but effects due to differences in genetic background cannot be excluded [29]. Moreover, it is generally known that retinoids influence many aspects of embryonic and fetal development [30], and a knockout of $Bcmo1^{-/-}$ during development might have caused part of the variation in the adult differences between both genotypes. To know whether this was the case, more research is needed, but until then results concerning the $Bcmo1^{-/-}$ have to be interpreted carefully.

The second question that was addressed in this study was: ''Do different tissues differ in response to a nutritional intervention?'' Effects induced by BC were highly organ specific and therefore analysis should focus on the individual target tissues. Nevertheless, surrogate tissues are sometimes used in nutritional interventions. An example is the use of PBMCs, which are readily accessible in humans. The use of PBMCs has proven to be successful for the profiling of nutritional changes [31] and as a diagnostic marker for certain diseases [32, 33]. Our study implies that results obtained in surrogate tissue should be validated in the target tissues before effects can be generalized and extrapolated to other tissues. This is especially important for functions that involve many different tissues, such as metabolism. Our data also show that, when properly validated, there might be some genes present after a nutritional intervention that are organ independently affected.

The last question that was addressed in this study was: ''Do female and male mice respond similarly to a dietary intervention?'' To our surprise, gene expression differences between male and female mice contributed most to the discrimination of individual animals in PCA in liver and iWAT, and resulted in the highest number of significantly regulated genes in iWAT. These differences have been described to be caused by sex hormones [34]. Also, the liver transcriptome was highly affected by sex. The liver contains also estrogen and androgen receptors [35] and several studies show that fluctuations in sex hormones result in differences in drug metabolizing cytochrome P450s [36]. The lung seemed to be less responsive to these sex effects compared to liver and iWAT but nevertheless, the sex effect was higher in lung tissue than effects induced by BC supplementation. A sex effect in lung is not illogical, since lung tissue contains androgen and estrogen receptors [37, 38] and several sex steroid-metabolizing enzymes [39]. Moreover, many sex differences have been reported for the lung, such as differences in the development and susceptibility to different diseases, such as asthma and lung cancer [40]. Interestingly, BC had also a large effect on the sex-dependent differences in iWAT of $Bcmo1^{+/+}$ mice and therefore might actually influence sex-specific effects. These results show that in BC research, but also in other molecular nutritional research, the interaction of sex and nutrition should not be neglected, especially in tissues such as iWAT and liver. It is therefore advisable to perform nutritional research, where differences are relatively small [41], in the sex of interest. Because the number of significantly regulated genes seems to depend on sex, these data also implicate that effects in vivo in one sex should be found in the other sex as well and that in vitro gene expression changes induced by nutrition are possibly difficult to correlate to effects in vivo.

Altogether, we aimed to answer three questions relevant for nutritional research: ''How many genes are changed by a change in nutritional intake compared to the number of genes changed by a difference in metabolism or sex and do they interact?'', ''Do different tissues respond differently and how does this translate to the use of surrogate tissues for mechanistic purposes?'' and finally ''Do female and male mice respond similar to differences in diets?'' We demonstrate that the effect of BC was generally much smaller than the effect of the knockout of *Bcmo1* and differences in sex, and that those effects were highly dependent on interactions with a functional Bcmo1 enzyme and sex (Table 1).

Table 1. Number of significantly regulated genes ($p<0.05$) induced by β-carotene (BC) supplementation, by knockout of *β-carotene 15,15′-mono-oxygenase 1* (*Bcmo1*) or by sex and the independency of these genes for these variables. The independency is measured as the overlap in genes that are regulated in both sexes (female and male), Bcmo genotype (Bcmo1$^{+/+}$ and Bcmo1$^{-/-}$) or diets (Control diet and BC diet)

					Regulated independent of						Regulated independent of
					Sex		*Bcmo1* genotype (BC metabolism)		Diet		
BC effect	Female *Bcmo1*$^{+/+}$	Female *Bcmo1*$^{-/-}$	Male *Bcmo1*$^{+/+}$	Male *Bcmo1*$^{-/-}$	*Bcmo1*$^{+/+}$	*Bcmo1*$^{-/-}$	Male	Female			Sex and *Bcmo1* genotype
Lung	651	1522	326	1474	20	89	14	32			0
Liver	732	871	323	653	13	50	10	29			0
iWAT	4970	1522	407	1202	131	33	33	90			0
Bcmo1 knockout effect	Female control	Female BC	Male control	Male BC	Control diet	BC diet			Male	Female	Sex and Diet
Lung	5585	5238	6341	5640	2893	2903			3529	2860	1957
Liver	5213	3952	6241	5195	2318	2113			3431	1991	591
iWAT	2487	4888	10017	9546	1268	2038			6284	943	411
Sex effect	Bcmo1$^{+/+}$ control	Bcmo1$^{-/-}$BC	Bcmo1$^{+/+}$ control	Bcmo1$^{-/-}$ BC			Control diet	BC diet	*Bcmo1*$^{+/+}$	*Bcmo1*$^{-/-}$	*Bcmo1* genotype and Diet
Lung	2251	2623	3330	2170			535	487	623	325	117
Liver	6716	6698	8709	6160			3828	3478	4238	4139	2377
iWAT	8403	13927	5802	6622			2537	4310	6437	2732	1617

Table 2. Number of significantly regulated genes ($p<0.05$) induced by beta-carotene (BC) supplementation, by knockout of *β-carotene 15,15′-mono-oxygenase 1* (*Bcmo1*) or by sex in lung, liver and inguinal white adipose tissue (iWAT) and the organ specificity of these genes

	Genes regulated in single tissue			Commonly regulated genes in 2 tissues			Commonly regulated genes in 3 tissues
	Lung	Liver	iWAT	Lung and liver	Lung and iWAT	iWAT and liver	Lung and liver and iWAT
BC effect							
Female *Bcmo1*$^{+/+}$	651	732	4970	10	106	203	4
Female *Bcmo1*$^{-/-}$	1522	871	1522	114	84	41	27
Male *Bcmo1*$^{+/+}$	326	323	407	3	4	7	0
Male *Bcmo1*$^{-/-}$	1474	653	1202	54	86	48	17
Bcmo1 knockout effect							
Female control diet	5585	5213	2487	1746	1212	974	710
Female BC diet	5238	3952	4888	1373	1293	1041	626
Male control diet	6341	6241	10017	2162	2803	2766	1261
Male BC diet	5640	5195	9546	1924	2307	2270	1033
Sex effect							
Bcmo1$^{+/+}$ control diet	2251	6716	8403	576	679	2255	240
Bcmo1$^{+/+}$ BC diet	2623	6698	13927	639	1085	3607	321
Bcmo1$^{-/-}$ control diet	3330	8709	5802	1084	708	1844	312
Bcmo1$^{-/-}$ BC diet	2170	6160	6622	514	542	1527	178

Moreover, the effects were highly organ specific (Table 2) and females and males responded differently to the effects induced by BC. We therefore conclude that mechanistic effects of BC should be investigated in the tissue of interest and that mechanistic effects in a specific sex cannot be extrapolated, without evidence, to the opposite sex. Moreover, effort should be taken to make sure the ''best possible'' genetic phenotype is used to answer nutritional research questions. Of course, it remains to be seen to which extent this conclusion can be translated to other dietary compounds, but at least it poses a warning that these factors should be carefully considered.

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The authors have declared no conflict of interest.

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