

Appendix 2
Workshop Booklet
**Increasing the Impact of European
Obesity Research: preparing for the ERA**

Workshop Booklet

Aberdeen, Scotland, UK, 12-14 January 2003

Scientific Steering Committee

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Programme

Sunday, January 12 2003

Time	Event	Room	Who?
1030 onwards	Registration	Ogston	All
1300-1500	Discussion/Briefing	Fraser	SSC, LOC, Fac., & Dis.Med
1615-1645	Light refreshments available	Ogston	All
1645-1715	Workshop Launch: Purpose & Process	Ogston	Dr. Julian Mercer Dr. Laura Meagher
1715-1815	Genomics & Biotech	Ogston	
1715-1745	Human Models		Prof. Johannes Hebebrand
1745-1815	Animal Models		Prof. Hans Joost
1815-1845	Tea/Coffee	Ogston	<i>All</i>
1845-1915	Improving Health through Diet	Ogston	Prof. Arne Astrup
1915-1945	Early Nutrition and Health	Ogston	Dr. Suzanne Ozanne
1945-2000	Looking to the Future with the EC	Ogston	Prof. Jim Leslie
2030-2230	<i>Dinner</i>	<i>Elliot</i>	<i>All</i>

Monday, January 13 2003

Time	Event	Room	Who?
0830-0900	Introduction to the Day	Ogston	Dr Julian Mercer Dr Laura Meagher

0900-1000	Breakout Session 1a		
	Genomics & biotech	Ogston	Group 1
	Improving health through diet	Elliot	Group 2
	Early nutrition & programming	Fraser	Group 3
1000-1030	<i>Tea/Coffee</i>	<i>Ogston</i>	<i>All</i>
1030-1230	Breakout Session 1b		
	Genomics & biotech	Ogston	Group 1
	Improving health through diet	Elliot	Group 2
	Early nutrition & programming	Fraser	Group 3
1230-1400	<i>Lunch</i>	<i>Dining</i>	<i>All</i>
1400-1530	Feedback Session 1	Ogston	All
1530-1600	<i>Tea/Coffee</i>	<i>Ogston</i>	<i>All</i>
1600-1800	Breakout Session 2		
	Genomics & biotech	Ogston	Group 1
	Improving health through diet	Elliot	Group 2
	Early nutrition & programming	Fraser	Group 3
1930-2130	<i>Dinner</i>	<i>Elliot</i>	<i>All</i>
2130-2230	Discussion/Briefing	Fraser	SSC, LOC, Fac., & Dis.Med

Tuesday, 14 January 2003

Time	Event	Room	Who?
0830-0845	Introduction to the Day	Ogston	Dr Laura Meagher
0845-1000	Feedback Session 2 (on Breakout Session 2)	Ogston	All

1000-1100	Breakout Session 3		
	Genomics & biotech	Ogston	Group 1
	Improving health through diet	Elliot	Group 2
	Early nutrition & programming	Fraser	Group 3
1100-1130	<i>Tea/Coffee</i>	<i>Ogston</i>	<i>All</i>
1130-1200	Feedback Session 3 (on Breakout Session 3)	Ogston	All
1200-1300	Plenary Discussion Wrap-up and Next Steps	Ogston	All
1300-1400	<i>Lunch</i>	<i>Dining</i>	<i>All</i>
1300-1430	Working Lunch – Discussion/Briefing	Elliot	SSC, LOC, Fac., & Dis.Med

SSC – Scientific Steering Committee, **LOC** – Local Organising Committee, **Fac** – Facilitators & **Dis. Med** – Discussion Mediators

Plenary Speakers - Position Papers

"Genomics and Biotech"

Speaker 1: Johannes Hebebrand

Title of position paper: Molecular genetic research in human obesity

In order to allow a fruitful discussion of future perspectives to be pursued in molecular genetic studies in obesity, we review the current "main stream" approaches to detect mutations and polymorphisms predisposing to the development of obesity and related phenotypes. We proceed by critically reflecting on these approaches to dissect both their strengths and weaknesses. Finally, from a subjective point of view we discuss complementary or alternative strategies which we believe will broaden our potential of understanding the genetic mechanisms involved in body weight regulation. The better our understanding of the genetic mechanisms and the underlying pathways becomes, the more we stand a chance of addressing genotype-genotype and genotype-environment interactions.

Phenotype: Apart from BMI percent body fat as assessed by bioelectrical impedance analysis, sum of thickness of different skin folds or other more sophisticated methods is frequently used as an additional phenotype in molecular genetic studies. Other variables correlated with both BMI and percent body fat are frequently co-determined. These include e.g. serum adiponectin and leptin levels.

Current phenotyping cannot address the major phenotypes energy intake and expenditure in sufficiently large samples. Instead, we usually measure anthropometric and endocrinological variables. Whereas these are undoubtedly relevant, it appears highly probable that the current phenotyping schemes may be missing out on other genetically based phenotypes that contribute to the development of obesity. In particular, behavioural and sensory phenotypes are currently not being analysed extensively in molecular genetic studies. This presumably stems from the fact that in medical terms obesity is frequently perceived as a metabolic disorder; this perception has led to a self-perpetuating mechanism in that the belief in a metabolic aetiology has facilitated access of clinicians and scientists into the field with a corresponding background who then use their expertise to study the phenotype. Behavioural phenotypes are frequently looked upon critically. However, it is frequently forgotten that even the most precise measurements of fat mass or serum parameters merely represent a momentary glimpse into a complex organism which is subject to developmental change.

The negligence of addressing behavioural phenotypes is becoming more and more apparent in light of the importance of central mechanisms in body weight regulation. Most of our current knowledge pertains to hypothalamic pathways. However, other brain regions have emerged and will continue to do so. As such, the implication of a specific region entails questions as to what behavioural and/or sensory phenotypes are related to it and if these might be relevant for obesity. For example, recent advances in understanding olfaction and gustatory sensation (1) lead to the question as to if and how genetic variability in sensation of smells and tastes have potential implications for eating behaviour and obesity. The brain reward system in which the nucleus

accumbens plays a prominent role is also a system that could have an influence on body weight regulation (2). Such considerations can also be extended to the candidate gene level. Thus, melanin concentrating hormone is exclusively expressed in neurons of the lateral hypothalamic area, that give off fibers to widespread brain regions including the olfactory bulb, anterior olfactory nucleus, neocortex and amygdala (3). A melanin concentrating hormone receptor 1 (MCHR1) antagonist has not only been shown to induce weight loss but also has both an antidepressant and anxiolytic effect (4). MCHR1 knock-out mice not only have an altered body weight but also hyperactivity (5,6). Conceivably, feeding-related functions of MCH include appetite, arousal and anxiety, food-searching behaviour, olfaction, regulation of energy balance, swallowing and mastication. In general, pathways involved in energy intake and expenditure might overlap with those relevant for mood regulation, activity, cognition and emotions.

This brief discussion serves to illustrate the fact that we could profit from an inclusion of behavioural and sensory phenotypes. Behavioural phenotypes generally show heritability estimates in the range of 0.5; as in obesity the environmental component is largely explained by non-shared environment. Behavioural phenotypes which warrant consideration for genetic analyses of body weight regulation include binge eating, activity level, drive for thinness, stress, mood and anxiety level (and their impact on eating behaviour). Furthermore, the delineation of taste and dietary preferences should prove valuable, too. More research is required to address which of these behavioural phenotypes has an influence on body weight. Depression which in prospective studies conducted children and adolescents has been shown to predict a higher BMI (6,7) may serve as an example.

Ascertainment of additional phenotypes/disorders: It is evident that not all conceivable phenotypes of potential relevance in body weight regulation can be addressed in every individual who is willing to participate in a molecular genetic study. Both the endurance of a proband and the costs for phenotyping represent a limiting factor. We recommend that behavioural phenotypes are better integrated into current assessment schemes. Alternatively, study groups well characterised for single behavioural phenotypes will be required to assess alleles once their involvement in weight regulation has been demonstrated.

It is trivial to point out that extensive phenotyping is prohibitively expensive and as such represents a limiting factor. It is debatable if the samples that are used for genome scans and mutation screens really need to be that well characterised. Furthermore, new findings might make it worthwhile to assess a phenotype that was originally not considered as being within the spectrum of relevant phenotypes. On the one hand, it can be speculated that the more specific subgroups become the more homogeneous the underlying genetic factors will turn out to be. Such a precise phenotyping might actually be a prerequisite for identifying genes in the first place. On the other hand it might make more sense in proceeding the other way around by first identifying genes relevant in weight regulation in a large but phenotypically not too well characterised study group to then proceed to test the effect of specific alleles in specific subphenotypes. The latter strategy would also at least partially circumvent the effect of testing several different phenotypes within a single sample (multiple testing). For BMI alone several different peaks have been reported, some of which have been confirmed in independent studies. Evidently, for the identification of the relevant genes in these peak regions no further phenotypical information is required.

In light of the potential overlapping of specific pathways it is of interest to reflect what

additional phenotypes are of interest for obesity research. To mention but a few it would be helpful to be able to assess the effects of alleles known to be involved in body weight regulation in depression, anxiety disorders, hyperactivity, eating disorders, picky eating, and high fat intake.

Ascertainment schemes: To both detect specific alleles involved in body weight regulation and to fully understand their functional role cases, controls, trios, concordant and discordant sibships and extended pedigrees have been ascertained by different groups world-wide in ethnically diverse populations. Populations include both children and adults. In addition, several large epidemiological samples and cohorts exist which can be used to follow-up on an allele of interest. In our opinion, this diversity is to be encouraged because it allows synergistic strategies. However, it should briefly be pointed out that the issue of genomic controls is important in case-control studies. The transmission disequilibrium test is frequently preferred if parents can readily be ascertained. The limitations of affected or concordant (discordant) sib-pair approaches need to be kept in mind; in particular, infrequent major genes cannot be picked up unless a sufficiently large number of such families are available. Furthermore, minor gene effects (alleles associated with small relative risks) also cannot be picked up with up to several hundred families, because allele sharing only minimally surpasses the expected rate of 50%. The need to ascertain large study groups encompassing well over 1000 cases (trios) and the same number of controls is emphasised.

Merging genetic studies in animals and humans: QTL studies have been ongoing for several years in rodents, pigs and cattle. A priority for future research into the genetic mechanisms involved in body weight regulation is to bring experts together who work on different species. The occurrence of a functionally relevant SNP in the pig melanocortin-4 receptor gene is just one example illustrating the potential inherent to this approach. Some QTL peaks identified in pigs map to homologous human chromosomal regions which have also been identified in linkage studies of human obesity. Quite possibly, the same gene(s) underlie these peaks, thus indicating conservation not only of genes and pathways but also of the mechanisms leading to genetic variability of body weight. An additional advantage pertains to the ease with which relevant phenotypes can be assessed in non-human species.

Candidate gene approach: The candidate gene approach has proven to be successful for obesity. Thus, the conservation of hypothalamic pathways in rodents and humans has certainly aided in determining suitable candidate genes. This particularly applies to those candidate genes originally identified in animal models. All the spontaneously occurring obesity mutations in mice have been found to harbour functionally relevant mutations in humans, too, or have led to the identification of a system/pathway in which other genes were found to be mutated (e.g. carboxypeptidase mutations in mice and prohormone convertase 1 gene mutations in humans). However, it should be pointed out that most of these mutations in humans were not detected within a classical mutation screen. Instead, specific endocrinological findings such as elevated proinsulin levels or hypoleptinemia led investigators to screen specific genes with a clear-cut *a priori* hypothesis. Functionally relevant mutations in the melanocortin-4 receptor gene (*MC4R*) currently represent the only exception to this rule; this is related to the fact that obesity due to *MC4R* mutations is not readily distinguishable from "normal" obesity. It is readily apparent, that mutation screens of larger samples are required to identify alleles involved in obesity not readily associated with a specific endocrinological or behavioural phenotype.

We perceive two major perspectives for the candidate gene approach which can certainly both apply at the same time: 1. Within linkage regions the candidate gene approach is frequently resorted to in complex phenotypes when the number of putative genes has been narrowed down. Undoubtedly, we should witness the success of this approach for specific candidate chromosomal regions for obesity in the near future. 2. Some candidate genes warrant consideration independently of whether or not they are localised within chromosomal regions of interest. Such genes are considered because they are involved in relevant pathways as shown in animal models or via other evidence. This approach should not be viewed as an alternative to the identification of the genes contributing to linkage peaks. Instead such studies are complementary because as illustrated above linkage studies cannot readily lead to the detection of infrequent relevant mutations or minor gene effects.

Validation of an obesity gene: Irrespective of the approach for the choice of a particular candidate gene we need to devise how to conclude that a specific allele indeed predisposes to obesity. The current literature abounds with positive association studies some of which have been followed up with negative or equivocal results. Many of the positive results must be viewed critically because multiple tests were performed to achieve the respective "significant" result. It is crucial that negative findings are also published. Every effort should be made to encourage researchers to publish negative results; furthermore, journal editors need to be aware of their responsibility. Potentially, specific journals might be required to competently deal with these issues. To allow a better interpretation of negative findings, the power of the study for a given (previously reported) effect should be stated.

We deem it important that positive findings are followed up in a systematic fashion thus enabling the scientific community to conclude whether or not the identified allele is indeed involved. For this purpose, defined population (epidemiological) samples could be referred to in addition to large trio samples to allow the TDT; meta-analyses should prove helpful. At some point a decision needs to be reached as to whether current evidence is sufficient to unequivocally conclude that a particular allele(s) is involved. A final decision on the epidemiological level should be based on an appropriate meta-analysis of all available studies.

This procedure should be formalised by for instance a committee whose task would be to rank candidate gene findings according to the empirical evidence from improbable to probable and finally to definite. Rules for determining this status need to be defined. Such a formalisation would entail many benefits, the foremost of which would be the separation of solid findings from false positive or equivocal findings. The committee would need to consider if ethnicity, gender, age and other variables have an impact on the respective association. Gene-gene interactions can reliably be assessed if the contribution of every single gene is without doubt.

False negative findings are also an issue of concern. Most current candidate gene studies suffer from a lack of power due to small sample sizes. Therefore, the power of these studies to detect a given effect should be stated for the negative finding. Another way of addressing this issue is to include relative risks and sample size calculations in association studies based on the premise that the observed non-significant difference in allele frequencies between cases and controls is indeed real. This will enable follow-ups on negative association studies. Estimations of allele and/or genotype specific relative risks and attributable risks should be presented in all positive association studies.

The solidity with which a particular candidate gene has been investigated requires

attention. In many studies only a single SNP was analysed, in others two or more SNPs and haplotypes were addressed. A systematic mutation screen of the coding region represents an attempt to detect all mutations within a candidate gene. Finally, the promoter region can also be screened. Nevertheless, even this arduous approach does not totally allow the exclusion of a particular gene; theoretically a regulatory sequence can have quite a distance from the gene. The methodology also warrants consideration; thus, mutation detection rates upon use of SSCA or other methods are not 100%.

We are already witnessing a commercialisation of molecular genetic findings in obesity. Diagnostic tests based on specific polymorphisms or mutations are available commercially (e.g. Internet). In our opinion, the consumer/patient should have access to molecular genetic testing after having been informed of potential implications. Clearly, only tests should be made available that pertain to polymorphisms or mutations whose functional relevance has been established unequivocally.

Linkage studies: As has already been pointed, genome scans performed in obesity have come up with some consistent regions. Fine mapping is ongoing; the TDT is of great value for detection of linkage disequilibrium. The next years will reveal to what extent single mutations or SNPs and haplotypes underlie these peaks. Furthermore, it seems possible that some of the peaks actually represent the combined effect of SNPs or haplotypes at more than one locus. The fact that gene and regional chromosomal duplications have occurred frequently indicates that gene clusters might indeed play a role.

Genotyping: The advent of high throughput technology offers the chance to genotype more than 10000 samples a day using Mallditof. Undoubtedly, scientific groups have already or will devise ways and means to obtain access to high throughput facilities. This in itself might turn out to become crucial for obtaining a competitive edge. A substantial proportion of the work will shift from lab work to computer work. Both the candidate genes themselves and the known SNPs need to be identified. Statistical comparisons need to be performed; in light of the vast number of candidate genes, SNPs and haplotypes, false positive results due to multiple testing will become exceedingly frequent; testing of SNPs will basically occur on a genome wide level (9). Appropriate decision rules and additional (high throughput) molecular studies in other samples are required to determine whether or not the polymorphism is to be pursued by for instance genotyping it in a second sample. Because "significant" findings will additionally require functional studies, efforts must be maximised to allow identification of those genes and alleles which are indeed involved in the phenotype. Specific guidelines for a sequential procedure entailing a high probability of identifying an allele with an impact on the phenotype could prove valuable in this situation.

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Speaker 2: Hans-Georg Joost

Title of position paper: The Need for a Focussed, Coordinated European Research Effort to Stop the Current Obesity Epidemic

Background - Because of the dramatic, worldwide increase of its prevalence and of its secondary complications, obesity represents the most important contemporary health problem (Flegal et al., 2002). It is the central component of the metabolic syndrome, or syndrome X, which comprises several obesity-associated abnormalities including dyslipidemia, insulin resistance, impaired glucose tolerance, and hypertension (Reaven, 1995). Most importantly, the syndrome is associated with type 2 diabetes mellitus and its life-shortening cardiovascular complications. During the last decade, the increase in the prevalence of obesity was accompanied by a parallel rise in that of type 2 diabetes. Thus, conservative estimates predict a doubling of the prevalence of diabetes within the next 10 years. It is generally agreed that obesity has a polygenic basis, but that nutritional and behavioural parameters (Groop and Orho-Melander, 2001) are important co-factors. So far, few gene variants have been found to be associated with polygenic obesity which can account for only a small portion of the syndrome (Ukkola and Bouchard, 2001).

The financial impact of the co-morbidity of obesity is enormous. In Germany, public health insurance companies spent 1.2 billion Euro (6% of their total budget for drugs) for blood sugar-lowering agents (Joost and Mengel, 2002), and a much larger amount for treatment of the total co-morbidity of obesity (estimated as 25% of the total budget). Given that we at present see only the beginning of the epidemic, and given that the current prevalence of obesity predicts a doubling of the prevalence of diabetes by 2010, the public health system in Europe faces a high financial crisis unless an effective prevention of obesity is established now.

In view of the dramatic increase of obesity, the current strategies for its prevention and treatment appear largely ineffective. Thus, the need for a new, successful effort to stop the current epidemic is obvious. This effort needs to be interdisciplinary, combining the expertise of genetics, molecular biology, nutritional sciences, and neurobiology in both experimental and clinical research.

Prevention and Treatment - Prevention and treatment of obesity is currently based on food intake-restraining and exercise-enhancing programs ('life style intervention'), and on treatment with appetite inhibiting drugs. In addition, surgical methods (gastric banding and gastric bypass) are rarely used options for the treatment of *morbid* obesity. Research is mainly focussed on the molecular basis of the disease, i.e. on the identification of predisposing genes.

The search for obesity genes: Knowledge of the genetic basis of obesity is not at all essential for counseling and prevention. It is undoubtedly required, however, to identify new targets for drug treatment (see below). Also, understanding the basic mechanisms will facilitate the design of novel treatment strategies.

During the past decade, the identification of obesity genes in rodent strains with **monogenic obesity** has greatly advanced our general knowledge of the neuroendocrine pathways that control food intake and body weight. However, identification of these genes has done little to advance the understanding of the *genetic basis* of **human** obesity. Furthermore, efforts to identify human obesity genes by linkage or association studies have so far been unsuccessful. Thus, although the efforts to search for obesity genes in **human populations** should be continued, a shift in focus – back to the mouse models - has to be considered. **Mouse strains of moderate or morbid polygenic obesity** may be valuable models for dissecting the complex combination of genetic effects, and for identification of the underlying gene variants (Plum et al. 2000, 2002; Reifsnyder et al., 2001).

Food preference: The current obesity epidemic is in part produced by the widespread consumption of a high-calory, high-fat and sucrose-enriched diet. Little is known as to why there is a solid preference for such an adipogenic diet, and novel approaches are needed to elucidate this basis of the preference for 'unhealthy' food. Such knowledge may lead to strategies to improve the palatability of low-caloric, low-fat food. Obesity research could therefore include the molecular basis and systemic physiology of chemoreception (taste and olfaction). Also, in mice the preference for macronutrients has a genetic basis, which offers another starting point to approach the problem .

Thermogenesis: Several lines of evidence indicate that obesity is due not only to increased food intake but also to a reduced energy expenditure. However, the differences between normal and obese individuals are minute and very difficult to measure. Thus, definitive proof and also in-depth knowledge of the mechanism of such an imbalance of energy homeostasis is lacking. In view of the importance of this point for an effective treatment, research on the role of thermogenesis should have a high priority.

Drug treatment: Treatment of obesity with appetite-inhibiting drugs has only short-term success. Long-term application of the currently available agents induces tolerance, and is accompanied by unacceptable risks. Thus, there is clearly a need for agents that safely and effectively inhibit appetite. These drugs may come out of current research

focused on known or recently identified targets e.g. NPY receptors, MSH receptors, orexin receptors, or ghrelin receptors. Identification of additional drug targets should be a priority (see obesity genes). In addition, a reasonable (although not new) target of treatment is the energy homeostasis, and a specific increase in energy expenditure should be very effective in reducing body weight.

Lifestyle intervention: Current and previous concepts and programs for weight reduction by 'lifestyle changes' have shown some effects in the short run, but are extremely disappointing in the long run. The major problem of all programs is that once the intervention is terminated, the body weight rapidly returns to the elevated levels (the so-called jo-jo effect). For obese persons, an enduring weight loss therefore requires a continuous intervention which is difficult to maintain. Also, during the intervention counterregulatory effects appear to impede the weight loss, reflecting the very tight regulation of body weight. The most recent intervention studies have therefore used a combined, cost-intensive approach of dietary and exercise counseling which may not be realistic as a general public health program (Tuomilehto et al., 2001). It will be extremely difficult to circumvent this problem by novel approaches, possibly by a reward/punishment system that has already been suggested in some countries.

Surgery: The options for surgical treatment of obesity have received little acceptance by the general public and by medical experts. This may be due to the (debatable) belief that obesity is a behavioural abnormality which should therefore be corrected by altered behaviour, and which does not warrant the surgery-associated risks. However, the surgical treatment seems to be very effective (Torgerson and Sjostrom, 2001; Greenway et al., 2002). Thus, this option should further be evaluated, in particular with regard to its risk/benefit ratio and to its long-term results and complications.

Co-morbidity of obesity - Type 2 diabetes mellitus and its life-shortening macrovascular complications represents the most important consequence of the metabolic syndrome. During the last years, several studies have shown that intervention by weight reduction or drug treatment can effectively reduce the progression of impaired glucose tolerance to overt diabetes mellitus (Tuomilehto et al., 2001; Knowler et al., 2002; Chiasson et al., 2002). Thus, future research to improve such a preventive therapy will undoubtedly pay off. Basic research should focus on the **molecular mechanisms of islet cell failure** which precipitates diabetes. Two different hypothesis have been put forward for explain this process. The **lipotoxicity hypothesis** postulates that fatty acids, as a consequence of reduced storage capability of adipose tissue, accumulate in β -cells, produce damage of mitochondria and subsequent apoptosis. Alternatively, the **glucose toxicity hypothesis** assumes that blood glucose levels, which are elevated because of impaired glucose tolerance and which usually produce β -cell hyperplasia, exert detrimental effects on the β -cell if diabetes genes convert the proliferative into a pro-apoptotic stimulus. Identification of the responsible genes and elucidation of their function will be the key to prove either theory, and may lead to improved strategies for prevention of overt diabetes.

References

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"Improving Health through Diet"

Speaker: Arne Astrup

Note: No position paper for this research area was produced. This was due to circumstances beyond our control.

"Early Nutrition and Programming"

Speaker: Suzanne Ozanne

Title: Position paper on "Early Nutrition and Programming"

Introduction

Obesity is a major problem in the developed world and an increasing problem in the developing world. It has been reported recently that in the U.S.A. 22.5 % of adults are obese (defined by a body mass index > 30) (Flegal et al., 1998) and even more alarming 11 % of children are now obese (Troiano & Flegal, 1998) and these figures are still increasing. Obesity is strongly linked to adult diseases such as type 2 diabetes and cardiovascular disease (Visscher & Seidell, 2001). The rapidly increasing amount of obesity is thought to a major underlying factor in the increasing prevalence of type 2 diabetes which it is predicted will affect 250 million people worldwide by 2020 (Songer & Zimmet, 1995). An enormous effort is therefore devoted to understanding the mechanisms by which obesity may arise and to the development of drugs targeted at reduce obesity. Decreased physical activity and increased calorie intake have both been implicated in the increasing prevalence of obesity. However, it is likely that different individuals will have different susceptibilities to these external influences depending on, for example, their genetic background and the environment experienced in early life. The following document will focus on the latter of these. It will start by summarising data suggesting that fetal programming may determine the detrimental effect of adult obesity and also data suggesting that appetite and therefore susceptibility to obesity may be determined by early nutritional experiences. It will finish by considering key priorities for future research.

Early Nutrition and Programming

A large number of epidemiological studies have demonstrated that there is a relationship between poor fetal and early growth and the subsequent development of adult diseases such as type 2 diabetes and the metabolic syndrome (Hales & Barker, 2001). This relationship has been observed in a wide range of populations world-wide thus there is little doubt that the relationship exists. However the mechanistic basis of this relationship and the relative role of genes and the environment remains the subject of much debate. It has been proposed that poor fetal nutrition leads to programming of metabolism in a manner beneficial to survival under conditions of poor postnatal nutrition (Hales & Barker, 1992) This would give rise to a thrifty phenotype in which the organism was adapted store carbohydrate. This metabolic programming was proposed to become detrimental if the fetus was born into conditions of either adequate or over nutrition and obesity occurred. It may also increase the risk of the development of obesity.

Interactions Between Poor Early Growth and Adult Obesity

The apparent conflict between early nutritional experiences and adult obesity has been demonstrated by a number of human epidemiological studies. A study of 64 year old men in Hertfordshire U.K., demonstrated that the individuals with the worst glucose tolerance were those who were born small and who were currently obese (Hales et al., 1991). Individuals with a high birth weight were relatively protected from the detrimental effects of obesity. Individuals who were born small but remained thin were also protected from diabetes. This would explain why in countries where there is chronic

malnourishment, the prevalence of the metabolic syndrome is very low. Studies of individuals who were in utero during the famine known as the Dutch Hunger Winter have also shown that for any current body mass index, glucose tolerance was worse in those individuals exposed to the famine in utero compared to those born the year before the famine (Ravelli et al. 1998). This meant that frank diabetes was generally only present at age 50 in those individuals who were malnourished in utero and were currently obese. The detrimental effects of poor early nutrition and adult obesity have also been shown in animal models. For example early protein restriction and adult obesity (induced by the feeding of a cafeteria diet) have been shown to independently and additively cause an increase in systolic blood pressure in rats (Petry et al., 1997).

Programming of Appetite

In addition to being key to the full expression of the Thrifty Phenotype, it is also possible that obesity itself is a manifestation of the Thrifty Phenotype. Several studies have linked low birth weight to changes in body composition in adulthood including increased central fat (Law et al., 1992) and reduced lean mass (Gale et al., 2001). There is some evidence that the timing of the nutritional insult may also have an impact on the future susceptibility to obesity. Studies of men who were exposed to the Dutch Hunger Winter in early life have revealed that those who were exposed to the famine during the first half of pregnancy were more obese at age 19. In contrast those who were exposed to the famine during the last trimester of pregnancy and in early postnatal life had reduced obesity (Ravelli et al., 1976).

The detrimental effects of poor antenatal growth are exaggerated by rapid postnatal weight gain. Rapid weight gain in infancy has been shown to be a risk factor for future obesity. Infants who were growth restricted in utero and underwent postnatal catch up growth between birth and 2 years of age have been shown to be fatter and to have more central fat than other children (Ong et al., 2000). The mechanisms underlying postnatal catch up growth are not well defined. However such rapid growth may be the consequence of programmed changes in gene expression that were established in utero. It has been shown in a rodent model that maternal protein restriction is associated with increased expression of insulin receptors which could drive post natal weight gain (Ozanne & Hales, 2002). It may also be a consequence of programmed changes in appetite.

Increased rates of postnatal weight gain have been associated with reduced satiety in small for gestational age (SGA) infants (as assessed by volume of milk consumed by bottle-fed infants (Ounsted & Sleight, 1975). Leptin is one of many potential candidates that could mediate these changes in appetite. It has been shown that cord blood leptin is inversely related to rates of growth during infancy and that SGA infants have lower leptin concentrations (Ong et al., 1999). One possibility therefore is that low leptin levels in SGA infants lead to reduced satiety and rapid postnatal weight gain. Recent studies have also suggested that breast-feeding is protective against obesity risk in later childhood (von Kries et al., 1999). Bottle fed infants are known to have higher total energy and protein intakes than breast fed infants (Heinig et al., 1993) so one possible explanation is that early breast feeding may affect subsequent appetite regulation.

Studies in rodents have also revealed that early nutrition can have long-term consequences on appetite. Early studies with rats where nutrition during lactation was manipulated by altering litter size revealed that reduced nutrition during lactation resulted in a permanent reduction in appetite (Winick & Noble, 1966). More recent studies have shown that severe calorie restriction during pregnancy leads to

hyperphagia in adult life (Vickers et al., 2000). These findings suggest that appetite can be programmed upwards or downwards depending on the timing of the insult and suggest that the early postnatal period may be a time window for targeted intervention.

Questions/Priorities For Future Research

1. What are the major factors underlying differences in fetal nutrition? Is it possible to intervene to increase birth weight? How do we ensure that the increased weight is lean body mass and not fat mass? Would this reduce obesity and/or decrease the impact of obesity on health?
2. What are the critical time windows for programming of appetite? Is there a time window during lactation for intervention when appetite could be permanently decreased? Could this be a drug-free way of regulating appetite?
3. What are the key molecules involved in the programming of appetite? What does fetal growth restriction and early postnatal growth restriction do to expression levels of known regulators of appetite? Can we use animal models of growth restriction to identify novel appetite regulators? Understanding at the molecular level is required and this may lead to the identification of novel drug targets for prevention of obesity.
4. What are the mechanisms involved in catch up growth? Can catch up growth be prevented? Can it be minimised by breast-feeding? Would this reduce childhood and adult obesity?

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Discussion Mediators - Position Papers

"Genomics and Biotech"

Mediator: Dominique Langin

Obesity was recognized as a major public health problem by WHO in 1998. According to the International Obesity Taskforce (www.ionf.org) , the number of obese adults has increased to over 300 million worldwide. Current data from individual national studies suggest that the range of obesity prevalence in European countries is from 10 to 20% for men, and 10 to 25% for women. Doubling occurs every 15 years. Obesity is defined as a disease in which health is adversely affected by an accumulation of excess body fat. Several diseases including type 2 diabetes, coronary heart disease, hypertension and cancers are frequent complications of obesity. The economic costs of obesity are between 2 and 8% of total health care costs in western countries. Available treatments to reduce weight are not satisfactory. The improvement in treatments of obesity and related complications requires a concerted action on a better characterization of physiopathological mechanisms and the identification of novel drug targets.

Obesity is all but a homogenous disease with a unique etiology . The increase in body fatness is accompanied by profound alterations of physiological functions that are partially dependent on the regional distribution of adipose tissue. Visceral and generalized obesity is associated with cardiovascular dysfunctions. Accumulation of fat in the chest wall restricts respiratory excursion and is related to sleep-breathing abnormalities. Upper body obesity, i.e. intraabdominal accumulation of adipose tissue, has been linked to the development of insulin resistance, hypertension, diabetes mellitus and hyperlipidaemia. With regard to the causes of obesity, the importance of a combination of genetic, environmental and psychosocial factors is now well recognized. A current view at obesity is a model in which susceptibility to obesity is determined largely by genetic factors, but the environment determines phenotypic expression. As this position paper shall introduce the discussion in the Genomics and Biotech research area, I will give a brief overview of recent development in the genetics and functional genomics of obesity. A paragraph will be devoted to the current status and strategies of the medicinal treatment of obesity. The final part lists proposals of directions for future research.

Genetics of obesity

The dramatic influence of genes is shown by monogenic forms of obesity. In humans, mutations in 6 genes have been described : leptin (LEP), leptin receptor (LEPR), proopiomelanocortin (POMC), prohormone convertase 1 (PCK1), the transcription factor SIM1 and melanocortin 4 receptor (MC4R). There are several interesting characteristics of the monogenic forms of obesity . These mutations influence the central nervous system control of food intake demonstrating the importance of the pathways and their potential as targets for drug development. The most frequent form of monogenic obesity is due to mutations of the MC4R gene that are found in 4 to 5 % of obese subjects and leads to massive obesity. As search for mutations spreads among European hospitals, this means a significant number of people and families to take care of. Extreme obesity is also found in complex syndromes such as Prader-Willi, Cohen, Alstrom, Bardet-Beidl and Borjeson-Forssman-Lehman. Except for the Barder-Beidl syndrome, genes have not yet been identified. The study of the genetic causes of congenital lipodystrophy has also a relevance for obesity research because it may bring data on adipose tissue development in vivo. Moreover, these pathologies are characterized by complications such as diabetes which underlying causes may be very informative for obesity research.

For common forms of obesity, heritability estimates range between 50 and 90% indicating that genetic factors account for a substantial portion of variation in human adiposity . These genetic determinants are multiple and interacting with each other and with the environment. Association and linkage studies are synthesized each year in the Human Obesity Gene Map . In 2001, 58 studies report candidate genes with positive findings and 33 human quantitative trait loci were derived from genome scans on a total of 81 positive linkages. To date, no gene has been formally identified for common obesity. An often overlooked point is the distinction between the genetic causes of excess fat accumulation, i.e. the development of obesity, and those that predispose to complications associated with obesity. However, the two aspects clearly correspond to distinct approaches in terms of therapeutic strategies.

The area benefits from a wealth of animal models either obtained by transgenesis or by selection of spontaneous variants . These models have provided

considerable insight into the biology that underlies fat mass regulation. Modern transgenic techniques with spatiotemporal control of gene expression will undoubtedly prove very useful. Other experimental strategies based on the quantitative trait locus analysis of genetic variations among inbred strains can be used to study the genetic basis of phenotypic trait. Such approaches combined with improved physiological techniques and functional genomics could be used on transgenic animals to characterize modifier genes.

Functional genomics of obesity

Several studies have been published using DNA chip technology in the context of obesity. Differences in gene expression have been identified in adipose tissue from lean and genetically obese mice . Cell culture models have been widely used to determine changes in the transcriptional profile during adipogenesis, the effect of drugs on adipocyte gene expression and differences between white and brown adipose tissue. In humans, the use of quantitative RT-PCR has boosted the development of in vivo studies on adipose tissue and skeletal muscle gene expression. The combination of microbiopsy, amplification of mRNA and sensitive labeling of cDNA probes now permits studies of adipose tissue and skeletal muscle transcriptomes in vivo .

To date, published data on proteomics in obesity are scarce .

Strategies in medicinal treatment of obesity

Two drugs are available in Europe. Sibutramine inhibits the reuptake of serotonin and norepinephrine. In clinical trials, it produces a dose-dependent decrease in body weight. Its side effects include dry mouth, insomnia, asthenia, and constipation. In addition, sibutramine produces a small increase in blood pressure and heart rate. Orlistat is the other drug approved for long-term use in the treatment of obesity. It works by blocking lipase and thus increasing the fecal loss of triglyceride. There is reduction of serum cholesterol that cannot be accounted for by weight loss alone. In clinical trials, it produces a loss of weight. Its side effects are entirely due to undigested fat in the intestine.

Despite a significant improvement in the metabolic risk factors, the two drugs are not the panacea. By analogy with hypertension, one can consider that more drugs with different mechanisms of action are needed for combined drug therapy. Needless to say that calorie restriction, physical exercise and behavioral therapy will remain indispensable. The current strategies to reduce fat mass have been recently reviewed . Reduction of food intake may be one goal. Much progress has been made in the understanding of the complex network of orixogenic and antiorixogenic factors. Several hypothalamic receptors appear to have good potential for pharmacological development. Interference with nutrient absorption is another possibility as demonstrated by orlistat. Stimulation of energy expenditure is an attractive solution especially if combined with a stimulation of adipose tissue lipolysis. Finally, fat metabolism and adipogenesis may be targeted.

Directions for future research

- One may infer from analogy with mature onset diabetes of the young (MODY) that, in addition to MC4R mutations, yet to be discovered genes contribute to frequent forms of monogenic obesity.
- Due to its relatively high prevalence and the lack of available treatment, therapeutic

strategies may need to be developed for patients with MC4R mutations. Linked to this goal is a characterization of the complications (or lack of) associated with this form of monogenic obesity.

- The identification of genes involved in pleiotropic syndromes in which massive obesity is a salient feature and in congenital lipodystrophy may unravel interesting and unexpected pathways that are important in the genesis of common obesity.

- Independent replication of linkage studies for QTLs with measurements of identical intermediate traits may save time and effort for the subsequent heavy task of gene identification.

- Studies to investigate the genetic basis of obesity-related complications among obese subjects need to be developed. These studies may help to determine whether gene-gene interactions confer a specificity to the diabetes or cardiovascular disease of the obese.

- Transgenic mice expressing Cre recombinase in specific tissues are indispensable tools for the production of tissue-specific gene knockout. Collaborative effort are needed to facilitate the generation and phenotypical characterization of genetically modified animal models of clinical-relevance. One can also envisage a coordinated plan for *state of the art* technology acquisition and development of new protocols-techniques aimed at studying whole-body and tissue metabolism in the mouse.

- In order to determine interindividual variations in gene expression, there is a need for low cost highly sensitive DNA chip to be used with materials from human biopsies. To get the full potential of microarray data, the development of novel bioinformatic tools (esp. for data mining) is mandatory.

- Combination of gene expression and genetic profiling with phenotypic characterization constitutes an important goal for obesity research.

- An adaptation of proteomics techniques is required to work on adipose tissue and skeletal muscle microbiopsies.

- The development and characterization of human preadipocyte and myoblast cell lines for in vitro study of gene expression and validation of novel drugs remains necessary.

References

"Improving Health through Diet"

Mediator: Wim Saris

Note: No position paper for this research area was produced. This was due to circumstances beyond our control.

"Early Nutrition and Programming"

Mediator: Berthold Koletzko

Title of position paper: Is there early metabolic imprinting of later obesity risk?

Obesity is now considered a global epidemic because its prevalence in both adults and children is increasing worldwide at an alarming rate (1–7). This increase is occurring not only in affluent countries but also in countries in economic transition and in developing countries. It has been related to an increasingly sedentary lifestyle with less physical activity, as well as to changing dietary habits (6,8–11). Obesity has marked adverse effects in childhood and adolescence as well as long term effects that extend well into adulthood. Obese children often experience psychosocial distress and, in many cultures, considerable discrimination. Obese adolescents are at a clear disadvantage with respect to completion of advanced education, household income achieved in adulthood, and rates of marriage (12,13). Obesity during childhood and adolescence affects the cardiovascular risk factors of dyslipidemia, glucose intolerance, and arterial hypertension.

Dyslipidemia, with increased concentrations of plasma triglycerides and low density lipoprotein cholesterol and reduced high density lipoprotein cholesterol, is a common finding in young obese individuals (14,15). Obesity causes reduced insulin sensitivity, pathological glucose tolerance, and increased fasting and postprandial blood glucose concentrations, and it appears to play a key role in the marked increase of non-insulin-dependent diabetes mellitus recently observed in some pediatric populations (16).

Other consequences of early obesity are non-alcoholic steatohepatitis, which is not always benign but may result in cirrhosis (17,18), cholelithiasis, pseudotumor cerebri, sleep apnea (which may be associated with neurocognitive deficits), disorders of the musculoskeletal system, and orthopedic complications with an increased long term risk of arthrosis, as well as polycystic ovary disease (15).

Persistence of childhood obesity into adult life is common. It has been estimated that between one third and two thirds of all obese children become obese adults, whereas about one third of adult obesity develops from obesity already manifest in childhood (19,20). The risk of persistent obesity increases with increasing age of the child, the degree of obesity, and the presence of parental obesity (21). Persistence of obesity during adult life is associated with markedly increased morbidity and mortality (22,23). It is of interest that childhood obesity is also reported to be related to an increased morbidity risk in adulthood even if obesity does not persist into adult life (24,25).

In view of these marked adverse effects of childhood obesity, the fight against this new epidemic has become a prime objective for pediatric health care (1,2,26). As available therapeutic interventions in obese children aiming at weight loss are costly and have less than satisfactory long term success rates, identification of strategies for effective prevention of obesity is particularly attractive.

IS THERE EARLY METABOLIC IMPRINTING OF LATER OBESITY?

Although genetic predisposition is of major importance for an individual's risk of becoming obese (27,28), the rapid increase in obesity prevalence in many parts of the world provides evidence for the strong modulating effects of exogenous factors. In addition to current lifestyle factors, events during early life appear to modulate later obesity risk, a phenomenon referred to as metabolic programming (29) or metabolic imprinting (30).

Indications for the existence of metabolic imprinting of obesity were provided by Ravelli

and coworkers, who studied a cohort study of 19-year-old men at military induction who had been exposed during perinatal life to the Dutch famine of 1944–45 (31). In this cohort, maternal exposure to famine during the last trimester of pregnancy and the first months of life was related to significantly lower obesity rates, while exposure during the first half of pregnancy resulted in a higher obesity prevalence than in non-exposed controls (31). A later follow up study of women and men at age 50 years who were either exposed or not exposed to famine in late, mid, or early gestation reported a significantly higher body mass index (BMI) in exposed than non-exposed women, whereas there was no significant difference in men (32). Also, children of Pima women with diabetes during pregnancy were found to have a higher risk of later obesity than children of mothers who did not suffer from gestational diabetes, which persisted after correction for other influencing factors (33). The findings cited suggest that metabolic perturbations of regulatory systems established in early gestation contribute to the development of obesity in later life.

In view of these results, we have recently addressed the question of whether prolonged breast feeding might have long term programming effects on the prevalence of overweight and obesity in children at school entry (34). In this study carried out in Bavaria, Germany, the risk for overweight and obesity was decreased by exclusive breast feeding. Here we aim to summarize the results of that study, discuss whether the apparently reduced risk for overweight and obesity in breast-fed children can be attributed to the properties of human milk, give further details on the lower prevalence of overweight/obesity in breast-fed children in Bavaria, and stimulate discussion as to which components of human milk might be instrumental in reducing the risk of obesity in breast-fed children

DOES BREAST FEEDING MODULATE LATER OBESITY RISK?

The study was performed as part of the Bavarian school entry health examinations 1997 enrolling 134,577 children (Fig. 1). From February 1997 to August 1997 some 13,345 children examined in two rural Bavarian regions (Oberpfalz and Niederbayern) and their parents were given a questionnaire on risk factors for atopic diseases. The overall response rate by the parents was 76.2%. The total number of completed questionnaires was 10,163. These data were linked to data on length and weight, measured as part of the routine health examination. The BMI was calculated as weight (kg)/height² (m). The analysis was confined to 5 year old (n = 1975) and 6 year old (n = 7382) German children, resulting in 9357 questionnaires for the analyses. The age and sex specific distribution of the BMI in all German children investigated during the 1997 school health examination in Bavaria was used as the reference to define overweight (BMI > 90th centile) and obesity (BMI > 97th centile).

The main exposure was exclusive breast feeding and its duration. The question on breast feeding was: "Was your child breast-fed?" If the answer was yes, the further question was: "For how long was your child exclusively breast-fed?" The categories offered in answering this question were: for not more than 2 months; 3 to 5 months; 6 to 12 months; and for more than a year.

In order to identify covariables potentially associated with breast feeding, several additional items were considered. These included housing characteristics and lifestyle (for example, the age of the house, whether the child had a separate bedroom, maternal smoking in pregnancy, spare time spent outside in summer and winter), questions on the child's health (prematurity, low birthweight), questions on diet (time of introduction of solid foods, consumption of own cooked food or industrial ready-to-feed products, food

bought in health food shops), and quantitative questions (never, less than once weekly, once or twice, three to six times weekly, or daily) on the consumption of selected dietary items (milk products, fish, meat, fat, carbohydrates). The highest education of either parent was used as a marker of social class.

The results show that the duration of breast feeding was associated with a progressive reduction in the prevalence of overweight—and even more so of obesity—in children at school entry (Fig. 2). Previously breast-fed and non-breast-fed children differed significantly in several indicators of social class and lifestyle. Many of these were also associated with overweight or obesity. High parental education, prematurity, and low birthweight were associated with a reduced risk of overweight and obesity, whereas maternal smoking during pregnancy was related to an increased risk. Full fat milk products (milk, quark or yogurt, whipped cream) and sweet deserts may be avoided by overweight children, suggesting a spurious "protective" effect of these products as compared to a "risk" associated with the consumption of the low fat version of these products. There was also an apparent reduction of the risk of overweight and obesity associated with a high consumption of butter and breakfast cereals (34).

Parental education was the only factor accounting for a > 10% shift of the odds ratio for breast feeding and overweight and obesity towards unity. Other factors which remained significantly associated with overweight/obesity in the final logistic regression model were high parental education, maternal smoking during pregnancy, low birthweight, having a separate bedroom for the child, and frequent consumption of butter (Table 1). The adjusted odds ratios by duration of breast feeding are shown in Fig. 3. Being ever breast-fed reduced the risk of overweight by more than 20%, and breast feeding for 6 months or more reduced the risk by over 35%. Even more pronounced effects were observed with respect to obesity (25% and 43%, respectively).

IS THE REDUCED RISK FOR OVERWEIGHT AND OBESITY IN BREAST-FED CHILDREN RELATED TO PROPERTIES OF HUMAN MILK?

With all cross sectional studies there is a risk of recall/information bias. Some misclassification of the duration of breast feeding is likely when mothers have to remember details of their child's earlier feeding. This misclassification, however, is unlikely to be related to the outcome, because the overt aim of the study was the search for risk factors of atopic disease. Selection bias is also unlikely—the return rate of the questionnaires was high and unrelated to the outcome measure (34). Random misclassification of the measurement of height and weight is probable, as it is difficult to ensure that all persons involved in the measurement in more than 10 different public health offices used exactly the same equipment in exactly the same way. This misclassification, however, is unlikely to be dependent on breast feeding, as that information was not known to the persons involved in the measurement.

Breast feeding was associated with various lifestyle and dietary factors documented as part of the study. With the exception of parental education, none of these was a confounder of the association of breast feeding and overweight/obesity. High parental education in breast-fed children, however, only partially explained the association of breast feeding and overweight/obesity.

Information on important risk factors for overweight which might be confounders of the presumed protection by breast feeding could not be assessed optimally. These included lifestyle/social class and genetic risk factors for overweight or obesity

Parental education may not be the optimal indicator of social class, but additional information is difficult to obtain in Germany because information on family income are not generally answered satisfactorily in Germany. Asking for family income may account for a selection bias by non-response although a potential impact of social class on health is not perceived by the German population.

Physical activity is certainly an important risk factor for overweight/obesity. As the questionnaire had not been originally designed for this purpose these questions were limited to "time for playing outdoors", which—though associated with breast feeding—was not associated with overweight/obesity and was not a confounding variable.

Diet is another important lifestyle factor associated with the risk of adiposity. The questions on this were confined to a semi-quantitative assessment of the present diet. Many overweight children, however, might have changed diet to reduce their weight.

Parents' weight is an important indicator of the genetic risk for overweight/obesity (27,28), and maternal overweight appears to be associated with a short duration of breast feeding or no breast feeding (35). A positive family history of adiposity was not a confounder of the association between breast feeding and overweight/obesity in a previous study (36). After the publication of our results, the issue was investigated in another prospective cohort study, the German multicenter allergy study (MAS) (37,38). In the MAS cohort, longer breast feeding was less common in overweight mothers, but maternal overweight was not a confounder of the association of breast feeding and overweight/obesity.

A strong argument against lifestyle factors explaining the observed protective effect of breast feeding comes from a study by Kramer on Canadian adolescents born in the 1960s (36). In that study a similar dose-related, protective effect of breast feeding on the later prevalence of overweight/obesity was found. If such a dose-dependent protective effect were caused by lifestyle factors associated with breast feeding, similar confounding factors should have to have been operative during the different time periods in different societies. In Kramer's study only 18.5% of the children had been breast-fed, compared with 56% our study, suggesting that the mothers who chose to breast feed their children in the 90s in Bavaria formed a different population from those who chose to breast feed in the 60s in Canada. The lifestyle in the early 60s in Canada was almost certainly different from that in Bavaria in the early 90s. Although it is difficult to rule out the possibility that unknown factors associated with the lifestyle of families of breast-fed children might play a causative role in the apparent protective effect of breast feeding, this does not appear likely.

The age and sex specific distribution of the BMI in all German children investigated during the 1997 school health examination in Bavaria was used as the reference to define overweight (BMI > 90th centile) and obesity (BMI > 97th centile). The 90th and 97th centiles in Bavaria are considerably higher than the widely used French reference values (39) (Table 2). Thus most children defined as overweight according to the Bavarian centiles would have BMI values above the 95th centile of the French reference values.

The lower prevalence of overweight/obesity in breast-fed children in Bavaria does not reflect a shift in the entire distribution of the BMI in breast-fed children as compared with formula-fed children. There is no shift of the mean but the upper tail of the distribution is "fatter" in non-breast-fed children. This has implications for other studies in that our findings might not be reproduced if only means and their 95% confidence intervals are

considered. The biological model to explain the observed protective effect on overweight and obesity should match a shift in the "upper tail".

As our study was cross sectional, we do not have data on the longitudinal evolution of BMI during the first 5 years of life in these children. However, such longitudinal data are available from the analysis of the prospective German MAS study (37,38). It is of interest that the BMI data from the MAS cohort show that differences between the subgroups which had been breast-fed for less than 2 months or for at least 2 months, respectively, only evolved after the fourth year of life—that is, at the time of BMI rebound (38). Thus other studies that observe children aged less than 5 years might not be able to detect any BMI differences related to postnatal feeding.

IS A PROTECTIVE EFFECT OF BREAST FEEDING ALSO DETECTABLE IN POPULATIONS UNDER DIFFERENT LIVING CONDITIONS?

In cooperation with drs. jana vigneroval and katerina osancova, national institute of public health, prague, czech republic, and dr. lida lhotska, ibfan/geneva infant feeding association, geneva, switzerland, we had the opportunity to evaluate data from a cohort of czech children studied with support by the internal agency of the ministry of health, czech republic (registration number 0268-3) (40). cross-sectional survey data were collected in 1991 on 33,768 school-children aged 6 to 14 years in the czech republic. the data were analyzed using multiple logistic regression analyses. the main outcomes chosen were overweight defined as bmi > 90th percentile and obesity defined as bmi > 97th percentile. the results of the analysis show that children who had ever been breastfed had a lower overall prevalence of overweight (9,3%; 95%ci 8,9 – 9,6%) than children that were never breastfed (12,4%; 95% ci 11,3 – 13,6%). similarly, the prevalence of obesity was clearly lower in previously breastfed children (3,2%; 95%ci 3,0 – 3,4%) compared to previously formula fed children (4,4%; 95%ci 3,7 – 5,2%).

The effect of breastfeeding on overweight/obesity did not diminish with age in children 6 to 14 years old and could not be explained by parental education, parental obesity, maternal smoking, high birthweight, watching TV, number of siblings and physical activity. Adjusted odds ratios for breastfeeding were 0.80 (95%CI 0.71 – 0.90) for overweight and 0.80 (95%CI 0.66 – 0.96) for obesity. Thus a reduced prevalence of overweight/obesity was also detectable in an Eastern European country at a time with living conditions influenced by a socialist society. This suggests that the protection conferred by breastfeeding is not related to factors associated with breastfeeding in Western European capitalist societies and makes it more likely that compositional aspects of human milk may play a causal role.

ARE THERE COMPONENTS IN HUMAN MILK THAT REDUCE THE RISK OF OBESITY IN BREAST-FED CHILDREN?

Features of human milk feeding that might account for a lower risk for overweight/obesity in breast-fed infants could include hormonal responses, bioactive factors in the milk, a lower energy intake, or a lower protein intake, all of which might have long term effects.

Lucas *et al.* (41,42) reported significantly higher plasma insulin concentrations in formula-fed than in breast-fed infants, which would be expected to stimulate fat deposition and thus affect the early development of adipocytes. Human milk also contains bioactive factors that may modulate tissue growth and development. Breast milk contains both epidermal growth factor and tumor necrosis factor α , both of which

are known to inhibit adipocyte differentiation *in vitro* (34).

Nutrient intakes of breast-fed and formula-fed infants differ. Recent data indicate that the metabolizable energy and protein intakes of breast-fed infants are considerably lower than previously assumed and significantly below those found in populations of formula-fed infants (34). These early differences in macronutrient supply might have long term effects on substrate metabolism. In longitudinal follow up studies, Rolland-Cachera and coworkers observed a significant relation between the dietary protein intake at the age of 10 months with later BMI and body fat distribution (43,44). These investigators proposed that a high protein intake in early childhood might predispose to an increased risk of obesity at a later age. Indeed, in animal studies the early protein availability during fetal and postnatal development was found have long term metabolic programming effects on glucose metabolism and body composition in adult life. Whether or not postnatal intake of protein or other substrates does indeed affect later body composition needs to be tested in properly designed prospective trials.

EUROPEAN COMMISSION RESEARCH PROJECT ON "CHILDHOOD OBESITY – PROGRAMMING BY INFANT NUTRITION (CHOPIN)"

The EU Childhood Obesity Programme is a prospective multicenter trial with the aim to investigate whether the protein content and protein/fat ratio in infant formula and complementary feeds has lasting effects on later obesity risk. A one year multicentre intervention trial starting in the neonatal period will be performed in five European countries (Belgium, Germany, Italy, Poland, and Spain) with different habitual total protein intakes from complementary feeds to increase the range of protein intakes and thus to improve the statistical power to test the "early protein hypothesis" (www.danoneinstitute.org/Euchildhoodobesity). With a total budget of 2.8 million €, the EU Childhood Obesity Programme will study, over the first two years of life, body composition, hormonal status, protein metabolism, and new, simple anthropometric markers of childhood obesity. In addition, the whole study cohort will be followed up until the ages of 8 years. If a relationship between early feeding characteristics and later childhood obesity risk will be confirmed, it will offer the possibility for obesity prevention, for improving dietary advice to parents, and for developing nutritionally improved dietary products for infants.

CONCLUSIONS

Based on the data from this cross sectional study enrolling almost 10,000 children, there is strong evidence that breast-fed children are less likely to be overweight or obese at school entry than formula-fed children. This effect reflects "fattening" of the upper tail of the BMI distribution for non-breast-fed children but is not caused by a shift of the mean BMI. There are some indications that the reduced risk of overweight/obesity in breast-fed children might be related to properties of human milk and not to different genetic or lifestyle factors in these children. However, at present we cannot exclude the possibility that confounding variables such as parental BMI or other factors associated with breast feeding might play a role. The ongoing prospective EU Childhood Obesity Programme will offer an opportunity to test whether macronutrient composition of early feeding has a role to play in modulating long-term obesity risk.

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Table 1: Factors associated with breast-feeding and overweight and obesity in 5 or 6 year old German children and their impact on overweight and obesity (only significant factors in a logistic regression model with breast-feeding included) * at least 10% change of the OR for breast-feeding and overweight/obesity

	Prevalence in		Overweight		Obesity	
	not breast-fed Children	Breast-fed Children	OR	90% CI	OR	90% CI

high parental education*	41.4%	66.7%	0.77	0.67 – 0.89	0.62	0.49 – 0.79
Maternal smoking In pregnancy	12.8%	4.2%	1.51	1.20 – 1.89	1.82	1.28 – 2.58
Prematurity	13.8%	9.0%	0.78	0.62 – 0.98	0.69	0.46 – 1.03
Birthweight < 2500 g	10.4%	6.6%	0.69	0.48 – 0.84	0.78	0.54 – 1.10
Own bedroom	45.6%	54.4%	1.19	1.03 – 1.37	1.22	0.96 – 1.56
Margarine >= 3 Times per week	35.3%	32.4%	1.22	1.05 – 1.41	1.21	0.94 – 1.56
Butter >= 3 Times per week	60.5%	69.2%	0.73	0.63 – 0.83	0.70	0.56 – 0.88
Full fat milk >= 3 Times per week	50.8%	59.6%	0.69	0.60 – 0.80	0.54	0.42 – 0.68
Low fat milk >= 3 Times per week	31.9%	28.8%	1.72	1.49 – 1.99	1.77	1.38 – 2.25
Full fat quark or yogurt >= 3 times per week	28.8%	36.1%	0.66	0.56 – 0.78	0.52	0.38 – 0.70
Low fat quark or yogurt >= 3 times per week	25.9%	23.8%	1.42	1.22 – 1.66	1.32	1.02 – 1.71
Whipped cream >= once per week	18.6%	24.7%	0.65	0.54 – 0.79	0.58	0.41 – 0.81
Breakfast cereals >= 3 times per week	25.6%	35.3%	0.80	0.68 – 0.93	0.76	0.58 – 0.99

Sweet deserts ≥ 3 times per week	54.4%	57.8%	0.84	0.74 – 0.97	0.8 2	0.66 – 1.03
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Table 2: Prevalence (95% CI in brackets) of overweight (BMI>90th percentile) and obesity (BMI>97th percentile) in German children aged 5 or 6 living in two rural Bavarian regions in relation to breast-feeding.

		Overweight %	Obesity %
Never breast-fed	(n=4,022)	12.63 (12.36 - 12.90)	4.48 (4.38 - 4.58)
Ever breast-fed	(n=5,184)	9.18 (9.02 - 9.34)	2.78 (2.73 - 2.83)
Breast-fed for:			
<2 months	(n=2,084)	11.13 (10.60 - 11.59)	3.79 (3.62 - 3.96)
3-5 months	(n=2,052)	8.43 (8.06 - 8.80)	2.29 (2.18 - 2.40)
6-12 months	(n=863)	6.84 (6.12 - 7.56)	1.74 (1.55 - 1.93)
>12 months	(n=121)	4.96 (1.14 - 8.78)	0.83 (0.16 - 1.50)

FIG. 1. Schematic depiction of the study population derived from the obligatory school health examination in Bavaria in 1997.

Participated in school health examination - length and weight measured
n= 134,577



Children in 2 rural regions, who received additional questionnaire

n= 13,345



Informative
n= 10,163

questionnaire

returned



German children aged 5 or 6 years

n= 9,357



Information on breastfeeding available

n= 9,206

Never breastfed, n = 4,022

Ever breastfed, n = 5,184

Delegates Response to the Position

Papers

"Genomics and Biotech"

Name: Jon Arch

Title: Comments of on position papers

I fully endorse the comments of both H-G Joost and J Hedebrand that studies in animals can give perspective and leads for studies in human obesity. Regarding studies in humans, my impression is that, without massive studies, we are more likely to make progress from studies of subgroups (which are more like mouse strains of morbid polygenic obesity [Joost]) than from studies of diverse human populations [see Hedebrand].

Pharmaceutical companies sometimes target 'metabolic' genes identified by global KO approaches, without knowing whether the brain or the periphery is where the gene is

exerting its effect. I therefore endorse the need for more sophisticated KO technology.

Should we address the role of industry relative to academia in target identification and validation? It seems to me that where industry is weak is in putting the role of a gene into context in the regulation of energy balance. For example, is it involved in tonic (leptin-driven) or episodic, homeostatic or reward systems? How will a particular intervention influence energy balance? Which obese subjects are likely to benefit most?

The genetic basis of leanness in high fat/energy consumers should be pursued.

I am dubious of the statement that appetite-inhibiting drugs induce tolerance. It is true that weight loss reaches a plateau, but is this not because patients reach a balance between the effect of the drug and counter-regulatory mechanisms that oppose the drug's effect? The test is whether there is weight gain when drug therapy is stopped. Perhaps the real problem is that intervention in any single pathway cannot have a large effect – except for those few cases where obesity is monogenic in origin.

Name: Aldona Dembińska-Kieć

Title: The Importance of standardized European genetic study on the metabolic syndrome

There are no standardized, coordinated and validated long-term pan-European studies on the prevalence of metabolic syndrome with its main symptom of obesity. If such studies were conducted, they would allow the analysis of time- dependent trends in the evolution of the environmental genotype/phenotype relationships. Therefore, the research of these issues should be initiated in the frame of Priority 1 and 5 of the 6th Framework Programme.

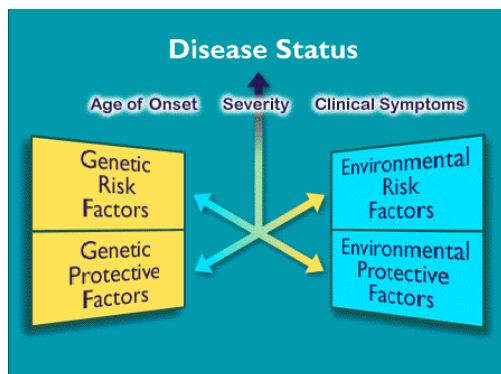
The EU's resources assigned for the investigation of the problems related to the metabolic syndrome have not been sufficient until now. Activities to define the magnitude of the epidemic of obesity in Europe and to introduce the appropriate measures preventing this illness are the tasks of the public health, which should be urgently implemented. This should be paralleled by the stimulation of basic research on mechanisms and genetic background of metabolic syndrome/obesity and its complications resulting in the development of the tools for early diagnosis as well as the promotion of life-style standards and functional foods for early weight control management in individuals at risk.

The risk of obesity is determined by the genetic-based susceptibility to environmental factors conducive to the development of this disorder. Several single gene mutations emerged from animal models of obesity, but human studies revealed a much more complex pathophysiology of this process. Human obesity results from the subtle interactions of the susceptibility genes with environmental factors favouring the deposition of excess calories as fat. It has been suggested that obesity is an adaptation of a "trifty genotype" to the environment with continuous availability of energy-dense, high fat/carbohydrate, easy accessible food, combined with low physical activity in individuals with genetically determined low capacity for fat/carbohydrate oxidation.

There is a growing clinical and experimental evidence concerning the number of genes and molecules participating in the control of feeding, energy disposal, adipose tissue development and metabolism, nutrient partitioning among tissues, and their metabolic relationships. Results of the studies lead towards a comprehensive molecular understanding of the body weight control system and are paving the way for new methods of obesity control, especially pharmacological, but also nutritional, possibly involving a local ethnic genetic background.

The main goal of the genetic analysis of obesity remains to identify the combinations of genes and their polymorphisms against the background of ethnic differences that contribute to human obesity/metabolic syndrome, and to define the environmental conditions under which they operate. Advances in molecular biology, proteomics and metabolic methods offer a powerful approach for detection of genes influencing the etiology of multifactorial diseases, such as the metabolic syndrome/obesity, and to study their interactions with environmental factors.

The increasing load of new information will require new methods and algorithms of data analysis. Replication of previous association studies can be performed in larger populations and in different ethnic groups. This needs an integration of international resources. Because of the late onset of complications associated with the metabolic syndrome, the phenotypic determination of obesity should be done with the special subset of statistical instruments. In order to strengthen the pan-European study experts who have been involved in longitudinal research of risk assessment for cardiovascular research (e.g. PROCAM study conducted by Prof Gerd Assmann's Group) should be involved. They can provide large DNA banks of a very well characterized populations. It can be helpful in the determination of genotype consequences at the time of the disease onset, and prediction the severity of clinical symptoms and complications.



The most important clinical consequences of the metabolic syndrome are vascular complications (hypertension, coronary heart disease, stroke, dementia), insulin resistance, type 2 diabetes, non-alcoholic fatty liver, cholesterol gallstones, gout, arthralgia, and some forms of cancer. They cause an increase of the health care costs of the growing percentage of the old population all over the world.

Abnormally increased levels of circulating free fatty acids, their metabolites and glucose in concert with certain hormones, autacoids and adipokines appear to interfere with the intracellular, insulin receptor-induced signaling system of muscle, adipocyte and hepatic cells explaining the pathophysiology of the excess of body fat and insulin resistance. Certain fatty acids, fatty acid derivatives and glucose are signaling molecules, which transcriptionally (through activation of peroxisome proliferator-activated receptors and

possibly other transcription factors) or post-transcriptionally regulate the expression of genes involved in the control of lipid metabolism, adipose tissue development, but also cellular differentiation, proliferation and apoptosis, thus in the regulation of organogenesis, inflammatory/immune reaction, etc. For example, low amount in diet of polyunsaturated fatty acids improves insulin sensibility, reduce lipid levels and cellular proliferation, and induce differentiation. It may explain low morbidity and mortality in cardiovascular disorders and certain types of cancer reported in populations with a high dietary intake of n-3 fatty acids. The ethnic related differences in gene polymorphisms of proteins (enzymes/hormones/autacoids and its receptors and intracellular pathways) involved in regulation of above processes is the one part and aim of experimental and clinical study.

A modern nutritional approach to weight control and obesity should take into account not only the energy and/or plastic properties of food components, but also their possible selective effects on the expression of obesity/(insulin resistance)-related genes and/or on the activity of the corresponding gene products. Nutrients with pro-thermogenic properties and/or capable to modulate adipogenesis, insuline resistance, adipokine production and secretion, could be useful in designing diets to help control body weight and/or the medical complications of obesity, and maybe the basis for the development of novel functional foods for weight control.

A good example is offered by vitamin A. In rodents, the vitamin A status plays an important role in the control of BAT and WAT function and development, with potential impact on body weight/adiposity. A poor vitamin A status favours an increment of adiposity that correlates with an increased WAT adipogenic potential and a depressed BAT thermogenic potential, while retinoic acid treatment and chronic vitamin A supplementation have essentially the opposite effects. However, the investigation of the unwanted side effects of the excessive supplementation should be in parallel conducted to avoid for example unexpected activation of immune system in gut or even the procancerogenic activity.

Then the development of rational and individually tailored therapy based on the individual genomic characteristic will be possible in the future for patients with the early diagnosed risk of obesity/metabolic syndrome detected by the modern molecular biology-based diagnostic tools.

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Name: Jörg Hager

Title: Response to Discussion Document "Genomics & Biotech"

Obesity is currently estimated to affect over 250 million people worldwide; a number that is predicted to continue to rise. The condition considerably reduces life expectancy, as it causes a dramatic increase in the risk of developing Type 2 diabetes, coronary heart disease, hypertension, osteoarthritis and certain forms of cancer. Indeed, in the US alone, an estimated \$100 billion are spent annually to treat conditions associated with obesity. As stated in the discussion document, in Germany alone health insurances spent up to 25% of their budgets to pay for obesity related impairments.

An ideal solution to this problem would be to tackle the cause of the obesity itself, rather than treating resulting disorders. Although companies have anti-obesity therapeutics on the market, current treatments based on impairing fat digestion by inhibition of lipase, or central regulation of metabolism and appetite, have not shown reliable long-term success. In addition some are associated with serious side effects, which include increased heart rate and blood pressure.

Genetic factors play a major role in the development of obesity. Identifying those genes and their respective pathways may enable the development of new therapeutics. The process, however, is likely to be complicated by the fact that obesity is classed as a complex disease. As such, scientists will have to identify a number of genes that increase a person's susceptibility to the condition, rather than a single gene defect. Moreover, as will be discussed below many genes causally implicated in obesity may not be valid drug targets.

Mapping obesity genes

Developments in molecular biology techniques have greatly increased our knowledge of diseases caused by single gene defects. The next challenge may be seen as genomic studies for complex diseases such as obesity, diabetes mellitus, asthma and coronary artery disease. This research is made much more difficult by the fact that these diseases are likely to involve a combination of genetic factors, rather than a single gene acting in isolation. There are several factors that are important for analysis techniques

used in gene mapping of complex diseases. These include speed, statistical power, accuracy and precision (e.g. how narrow is the locus identified) and also the size of the study population required. Reliability is also important in order to keep false positive rates low.

Biotechnology & Obesity

Biotechnology companies breach the gap between academic research and the drug development within the big pharmaceutical companies. Their research efforts often start well upstream than is possible for a big pharma company but as commercial entities they are usually more focused in their approaches towards drug development as is the case in academic institutions.

As pointed out in the discussion paper identifying the genes underlying the development of obesity may open the way to new treatments. However, one has to be careful when making such statements. A gene in itself is not a drug target in pharmaceutical terms. As a matter of fact most genes will likely turn out not to be useful as drug targets at all. The protein classes that can successfully be targeted by pharma companies are very restricted. The most promising proteins are within the families of cell surface receptors especially G-protein coupled receptors (GPCRs) and tyrosine kinase receptors. But even those are no guaranty for success. Two of the most promising targets for new treatments for obesity have been the neuropeptide Y receptors 1 and 5 for which involvement in feeding behaviour has been shown in animal models. Almost all of the major pharmaceutical players involved in obesity research have had projects in developing antagonists for these receptors hoping to suppress excessive food intake by obese people. However, most efforts on these receptors have recently been stopped because of insufficient effects at physiological reasonable doses of the antagonistic molecules.

One issue that may also hamper such development may also be the fact that pharma companies usually work on the wild type receptors while in obese people there may be variants that may impair or otherwise influence the correct function of the gene products. To identify those variants will be one issue for genetic studies.

In general all that is said above for human studies is in principle also valid for animal models of obesity. Although it might be easier to identify obesity genes (it has however to be noted that except for the monogenic mouse models that led to the identification of the first obesity related genes little progress has been made in identifying genes in polygenic mouse models of obesity) there is no guaranty that those will be drug targets in humans. Indeed the history of targets coming from animal models may make one rather pessimistic. Most have failed to be developed into new drugs, although often at later stages of the development (pre-clinical, phase 1 to 3 of clinical trials).

All the above should lead us to carefully evaluate the results from genetic studies. From a biotech or drug development point of view considerations about the "druggability" of a gene should be considered when deciding what genes or regions to study be it in humans or animal models.

Name: Jan Kopecky

Title: Adipose tissue metabolism as a target in the strategy for obesity treatment

Prevention and treatment of common obesity (see *Joost and Langin*) is based on affecting both sides of energy balance equation (i.e. energy intake and energy

expenditure), namely at the level of whole organism (i.e. food intake and physical exercise – "life style intervention"). Lack of success of the current strategy reflects the existence of a complex control of body fat content, the control which favors energy storage over energy expenditure. Development of such a control during evolution was enabled by establishing regulatory mechanisms at many levels. Thus, hypothalamic pathways affect sensory and (consequently?) behavioral phenotype (see *Heberbrand*) including food preference (see *Joost*), as well as metabolism of peripheral tissues and the efficiency of energy conversion (i.e. thermogenesis and energy dissipation). In turn, peripheral tissues affect the activity of hypothalamus *via* their own signalling molecules, with leptin representing the most important one. Leptin produced by fat cells controls adiposity as well as lipid oxidation in muscles and other tissues by acting both centrally and directly at the periphery (i.e. the control of metabolism of peripheral tissues is to a certain degree "autonomous", and independent on the activities of the hypothalamic pathways). This complex system of body weight regulation is endowed with many possibilities for counter regulatory effects that are difficult to overcome (the "jo-jo effect" - see *Joost*).

The strategy for treatment of common obesity may be improved in two ways:

- a. In order to enhance weight reduction, the treatment should affect in parallel several metabolic pathways contributing to fat accumulation. This would require to enlarge the current "complex" strategy (including life style, as well as drugs like Orlistat and Sibutramin) by including novel pharmaceuticals that would affect lipogenesis, fat oxidation, glucose metabolism, and thermogenesis by targeting specific metabolic reactions in liver, muscle and white adipose tissue (see below). It is apparent that metabolic pathways targeted by this obesity treatment may not be identical with those underlying common obesity. (b) In order to eliminate the "jo-jo effect", it is important to find the way(s) how to decrease adiposity without changing the signalling to hypothalamus (the signalling mediated by e.g. leptin and other, possibly yet unknown biologically active molecules secreted by adipocytes).
- b. It is evident that metabolism of white fat becomes an attractive target for novel drugs for the obesity treatment. Under all circumstances, changes in body fat content are eventually reflected by changes of lipid metabolism of adipocytes. Many candidate genes for obesity have important functions in white fat (1) and several models of mice exist that are obese or resistant to obesity due to transgenic modification of white fat (2). Our studies indicated that it is feasible to induce obesity resistance in transgenic mice by mitochondrial uncoupling protein 1 (UCP1) synthesized ectopically in white fat. Importantly, UCP1 depressed adiposity not only due to stimulation of thermogenesis, but namely by altering energy status (lowering the intracellular ATP/ADP ratio) of adipocytes, resulting in a complex change of lipid metabolism in white fat, including depression of *in situ* lipogenesis. Due to the link between energy status (mitochondrial energy conversion, mitochondrial uncoupling, and thermogenesis) and lipid metabolism in fat cells, the development of drugs with properties similar to those of the chemical uncoupler of oxidative phosphorylation 2,4-dinitrophenol [used extensively for the obesity treatment 70 years ago, but abandoned for the side effects of the therapy; ref. (3)], but acting specifically on adipocytes, will improve dramatically the obesity treatment. At the same time, energy status of adipocytes may represent a target for synergistic effects of various treatments that depress

adiposity by inhibiting *in situ* lipogenesis. With this respect, the mechanism by which food restriction, hypolipidemic drugs like bezafibrate, leptin, or long chain n-3 polyunsaturated fatty acids (n-3 PUFAs) and their non-metabolizable analogues inhibit lipogenesis and reduce adiposity should be clarified.

"Functional foods" affecting gene expression and metabolism in adipose tissue, liver and skeletal muscle should be further characterized, also with respect to their effectiveness during various phases of postnatal development. Based on this knowledge, composition of the diet should be optimized, with respect to both, obesity treatment of adults and children, and the "metabolic imprinting" in newborns (see *Ozanne* and *Koletzko*). In this respect, not only macronutrients (like proteins), but also "functional foods" (like n-3 PUFAs) must be considered.

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Name: Philip J. Larsen

Title: Fat animals will never be fat humans – and only few of them are good substitutes.

Human obesity is rarely resulting from single gene mutations. Most scientific authorities agree that the epidemic of human obesity currently sweeping most human populations first and foremost is caused by relatively sudden changes in their ways of living. Susceptibility of a population to develop disease depends to a large extent on the genetic background. However, there is little doubt that selection pressures today are substantially different from those of 10,000 years ago. As suggested by Jim V Neel, in his "thrifty genotype" hypothesis, modern society susceptibility to diseases such as diabetes has become deleterious consequences of genotypes, which had formerly been advantageous in human ancestral environments (Neel, 1962, *Am J Hum Genet* **14**:353-362). When it comes to store excess energy, it seems evident that cultural changes accompanying the progression from tribal society to urbanization have created novel environments for selection of genes involved in metabolic regulation rendering those genes favouring individuals to withstand meagre seasons disadvantageous.

During the last few years, the scientific community has been gifted with full-fledged keys to both the human genome and the genomes of several of their favourite experimental animals. The post-genomic research has experienced phenomenal technological progress, enabling scientists to screen large samples for genetic variation as well as occasion dependent expressions of both known and unknown genes. Also, it has become possible to identify virtually endless post-translational modifications of gene products even in very small tissue samples. With these powerful tools at hand, it should be possible to identify clusters of gene products responsible for the hyper-efficient regulation of human metabolism causing our populations severe problems. Furthermore, with the identification of gene products playing key regulatory roles in energy homeostasis, a panel of drug targets becomes available for the development of

anti-obesity therapy as well as alleviation of accompanying metabolic ailments.

Unfortunately, the ease by which post-genomic era technologies are applied to drug target identification is also the Achilles' heel of such discovery strategies. Microarray based identification of phenotype dependent gene expression has made it possible to compare transcriptional levels of virtually all genes in multiple tissues at several time points. Even the best available bioinformatics team, will face dire problems extracting the cluster of genes truly representing the endogenous regulator of energy homeostasis. This is partly due to the poor signal to noise ratio of microarray based "transcriptomics", but more importantly, good animal models mimicking the human obesity epidemic are hard to get. The overall conduction of energy homeostasis is governed by neurones of the hypothalamus, which via intricate neuronal pathways regulate such diverse actions as hunger, appetite, feeding, energy expenditure, and thermoregulation all of which have an impact on adiposity. Thus, in our study of environmentally induced changes of adiposity, it is pertinent to have access to CNS tissue samples further emphasising the need for good animal models.

Once such animal models are available, the quest seems quite simple. Despite several claims about the opposite, I will argue that very few animal models are available that reliably mimic the epidemic of obesity currently affecting human populations all over the world. Thus, several reports of phenotype dependent differences of gene expression are based on the genetically modified animals exposed to various environmental challenges (eg high-fat feeding). Sadly, most often the different phenotypes are due to differences in strain background rendering subsequent transcriptional analysis of little value. Other studies have used certain inbred strains (typically mice) characterised by their proneness to develop obesity. Such strains probably represent good models for testing anti-obesity drugs, but they constitute a poor tool to identify clusters of obesity causing genes because they represent outliers of a larger out-bred population. When searching for clusters of obesity causing genes in a population it is important that both those causing leanness and obesity are represented. An animal model of diet-induced obesity characterised by presence of both obesity prone and diet resistant rats have been developed by Barry E. Levin (Levin et al., 1997, *Am J Physiol* **273**:R725-R730). The applicability of this animal model to identify key regulators of energy homeostasis as well as anti-obesity drug targets will be presented at the workshop.

Name: Wolfgang Meyerhof

Title: Response to ERA position papers

Background

I understand from and agree on the position papers that there is general consent about the existence of an increasing worldwide serious threat to the health of human individuals caused by obesity and its associated disorders and to the public health care systems as well. Therapeutic opportunities are limited. Due to the conservation of pathways, studying monogenetic animal models also allowed deep insight into the neuroendocrinology of body weight control of humans. Human obesity, however, appears to be a multifactorial disease with a strong genetic component, but to which behavioural and probably sensory components contribute.

Required Research Activities

"Obesity Genes": Drs. Hebebrand, Joost and Langin have correctly pointed out that

the search for "obesity genes" undoubtedly represents a major research goal. Both systematic human genetic analyses and mouse genetics are indispensable for their identification. But I also think that other experimental strategies offer valuable alternatives. These include differential screening protocols with RNA preparations from (hypothalamic or adipose) tissues of control versus obese animals (MSG, PNO, diet-induced) and gene profiling methods. Candidate genes must be verified. Their function will have to be elucidated at the cellular, tissue and whole-animal levels. Polymorphisms in the candidate genes must be identified. We need to find out whether or not they are functional and to establish the association with BMI. Finally, their suitability for drug target needs to be examined.

Energy intake: Another aspect of the position papers is that, as obesity results from excessive energy intake, the homeostatic and hedonic mechanisms underlying feeding behaviour should be investigated. With regard to the former, research should focus on the activation/inhibition of neuronal circuitries, and the neurophysiological consequences thereof, in the central nervous system by peripheral factors derived from adipose tissue and the GI tract. The susceptibility of these networks to programming clues, which are mentioned in the papers by Drs. Koletzko and Ozanne, provides another interesting research opportunity.

So far the hedonic control of body weight regulation has attracted little attention. However, it is evident that feeding has a strong hedonic component, which represents a major determinant for the drive to feed (Saper et al., 2002, *Neuron* 36, 199-211). Drs. Joost and Hebebrand both mentioned this component, which results, at least in part, from the taste and smell of food and involves the brain's reward system. Identification of taste and odorant receptors and their transduction mechanisms as well as the coding and transmission of chemosensory information to the orexigenic and reward networks acquire attention. Linkage of gene polymorphisms to human psychophysics and the impact on BMI could be of interest as well.

Thermogenesis: I share the view of Prof. Joost that research on thermogenesis should be followed up. Thermoregulation involves central and peripheral mechanisms. It appears to me that the central mechanisms have been studied largely in the context of fever. Research at peripheral sites has to consider differentiation of brown and white adipose cells (see: Picard et al., 2002, *Cell* 111, 931-941).

Intestinal Absorption: Surgical and drug intervention and is successfully used to limit intestinal absorption. Orlistat targets fat absorption. Theoretically, another possibility would be to extent uptake inhibition to the other macronutrients.

Name: Thorkild IA Sørensen

Title: Obesity in the ERA

There is no doubt that the causes of obesity include genes and environment. The role of genetic predisposition has been convincingly proven in numerous twin studies and in complementary adoption studies, and the pattern of distribution of obesity within the families indicates that many genes are involved, possibly as various mixtures of genes with small and major effects and with gene-gene interactions. The rapid changes in occurrence of obesity within and between populations, in which there is no reason to suspect that changes in genetic predisposition, shows the importance of the environment. There is no contradiction between, on one hand, a strong genetic predisposition leaving little room for environmental influences on the within-population occurrence of obesity and, on the other hand, different environmental exposure resulting in major

differences in occurrence between populations or changes in occurrence within populations over time. However, in spite of enormous research efforts, the specific knowledge about which specific genes and which specific environmental factors are operating in common obesity is still scarce.

Apart from the consistent finding of an excess number of genetic variants of the MC4R in 2-5% of the obese, analysis of hundreds of genes have produced only weak and inconsistent indications. The complexity of the biology of development of obesity requires use of new strong genome-wide tools to find the genes at the genomic level as well as at the functional genomic level.

The prevailing paradigm assumes that the environmental causes of the obesity epidemic are operating either via an increased intake of energy, predominantly as food with high energy-density, and/or a reduced energy expenditure due to less physical activity both at work and in leisure time. It is surprising so little and weak evidence we have to support this common belief. There is no doubt that the recent changes in societies worldwide – the Westernisation – facilitate and even promote changes in the conditions of the intake/expenditure side of the energy balance equation, but when it comes to the link of these factors to occurrence of obesity, we are still on very soft grounds. When obesity has developed, the greater energy turnover creates a need for more energy, which may be satisfied by increased intake of particularly energy-dense food, and the physical activity may be reduced for several obvious reasons. However, from the epidemiological point of view, there is no convincing evidence demonstrating that such changes precedes the development of obesity from the non-obese state. Fairly strong epidemiological evidence suggests that at least one environmental factor operating in the first years of life plays a crucial role in setting predisposition to develop obesity in later life, a factor that does not necessarily manifest itself in obesity in the early life.

The accumulation of fat in the body reflects a tiny – still not measurable – positive energy balance, i.e. that intake has exceeded expenditure during the accumulation phase, but this thermodynamic law has no implications with regard to the direction of the process. So, a primary accumulation of fat that subsequently leads to adjustment of intake relative to output is as plausible as primarily increased intake or reduced expenditure. Thus, there are obvious needs to study the biology of fat accumulation in adipose tissue during development of obesity and during regain after weight loss.

It is often assumed that the fact that both genes and environment are involved in the causes of obesity implies that there is a gene-environment interaction, but if interaction means interdependency and not just co-occurrence, then it remains an open question. In humans, the contention is very vague and not supported by specific evidence, mainly because hitherto it has not been possible to study this type of interaction by appropriate methods. For such study to be informative, it should address possible interactions between specific genotypes and specific environmental exposures, and, when feasible, experimental design is preferable to observational design (see the NUGENOB poster or www.nugenob.com). Obviously, this approach will also strengthen the search for the specific genes and environmental factors that determines the development of obesity.

Name: Janet Treasure & David Collier

Title: Response to the "Genomics and Biotech" research area

The Western lifestyle is setting the backdrop for a natural experiment, which tests the control of eating behaviour, weight and body composition in a changing human environment. It has revealed weaknesses in the systems for setting upper limits to weight and appetite control for a large part of the population. Thus the atypical phenotype is for leanness and constrained eating behaviour. In the attempt to understand this modern vulnerability it may be helpful to examine those who show this unusual resilience. If we move to the pathological extreme, then anorexia nervosa, which has a strong genetic predisposition, may have important clues to this conundrum.

What side of the balance sheet are we interested in?

Many of the genetic causes of obesity, which are now being described result from abnormalities in the central control of appetite with overeating as a consequence. For example, one of the commonest monogenic causes of obesity relates to mutations in the melanocortin receptor, which has a central function in the hypothalamic adipostat. This means that phenotypes of obesity that focus on abnormal eating behaviours may be more pertinent than those with a focus on metabolic abnormalities.

Eating behaviours run in families. In a study on the Amish the heritability for disinhibition of eating was found to be 40%, and 30% for restraint. In a longitudinal study of eating behaviours continuities in attenuated eating behaviour persist over childhood. For example, poor sucking in infancy is associated with picky eating at ages of 3.5 and 5.5. There is some evidence that suggests that picky eating or eating problems in childhood predispose to anorexia nervosa or later eating disorders. The mechanisms underpinning these effects are uncertain. Extreme eating behaviours may be caused by increased hypothalamic drive but also they may be moderated by variations in olfactory and gustatory sensory perception or in the reward system or in aspects of personality (childhood obsessive compulsive traits powerfully predict the onset of eating disorders).

What is the state of the art in the genetic mechanisms for the eating disorders and eating behaviours?

Family and twin studies indicate an increased risk of both AN and BN in relatives of AN and BN probands. In addition, subthreshold forms of eating disorders appear to lie on a continuum of liability with full eating disorders. Multipoint linkage analysis using the broad diagnostic category of AN/BN did not yield evidence for suggestive linkage in the entire genome. However, analysis of a much smaller subset of kindreds (n=37), characterized by those pedigrees with at least one pair of relatives with the narrow phenotype of ANR, yielded multipoint non-parametric evidence (Z-all score of 3.0) for linkage to chromosome 1p34. Two quantitative variables drive for thinness and obsessionality (EDI-2 DT and Y-BOCS) tracked most closely with anorexia nervosa and there was also suggestive linkage to chromosome 1, 2 and 13. Bulimia nervosa and self induced vomiting has been found to be linked to chromosome 10. However the areas of linkage of the various eating behaviours in the Amish did not relate to any of these regions found in pathological eating.

What are the effects of developmental milestones?

Puberty is a key transition in body weight and appetite control with enormous changes in growth and body composition. It is noteworthy that this is the age at which eating disorders begin. In studies of eating behaviours in twins the shaping of the phenotype by genes and the environment undergoes a step change at puberty. Shared environment had an effect in the prepubertal twins, but this effect disappeared at puberty when additive genetic influences became the dominant contributor to the variance in eating disorders scores . It will be interesting to examine the eating behaviours of children with some of the monogenic causes of obesity caused by central mechanisms and to examine whether there are any changes in behaviour over puberty. Other developmental transitions such as motherhood may also be significant.

What are the other environmental exposures that can disturb eating behaviours?

Parental neglect greatly increased the risk of developing obesity in comparison with harmonious support (Odds ratio 7.1 (95% CI 2.6-19.3) . This finding was replicated in an additional longitudinal study which found that childhood adversity predicted the development of the bulimic disorders .

What new technologies may be of help?

Neuroimaging has demonstrated that the human central appetite control system is comprised of an orexigenic (i.e. appetite promoting) network and an inhibitory control (or anorexigenic) circuit; the balance between these two subsystems determines eating behavior and people with eating disorders have abnormal activation . Further research which links functional neuroimaging with research into genetic and childhood risk factors is needed to develop these ideas further.

Conclusions

Thus genes and various types of general and specific environments predispose to the development of a disturbance in eating behaviours. Many questions remain. We would endorse the need to describe the various phenotypes in greater detail. This will include behaviours and personality traits in addition to various biological markers. We argue that it is important to look at the more atypical phenotypes such as leanness and anorexia nervosa to understand more fully the disorders of appetite, weight and body composition.

Name: Hubert Vidal

Title: Delegate response on "Genomic and Biotech" research area

First I would like to thank Drs. Joost, Hebebrand and Langin for their excellent position papers that clearly and correctly reflect the situation and that identify adequately key priorities for future research. The aim of this response is only to put more emphasis on some aspects of the "functional genomics" approach in obesity. Based on my experience in gene expression analysis in human adipose tissue and skeletal muscle, using quantitative RT-PCR, cDNA microarrays and more recently proteomics, I suggest that some coordinated efforts should be dedicated to the following issues:

1- The validation and identification of "reference" (or standard) methodologies. This is particularly important for the DNA chip technology, but it also applies to the other approaches. Several methods can be used and little has been done to demonstrate the reproducibility and the accuracy of these different approaches. Methods for RNA preparation, amplification and labelling should be also optimised. Up to now, there are

no recognized reference methodologies and I think that such selection should be done at the European level.

2- The elaboration of (large) international tissue banks. In addition to the methodologies, that are not yet completely validated as pointed out before, the determinant parameter of all approaches in functional genomics is the quality of the tissues or samples that will be analysed. It should be important to define, at the European level, the requirements for setting up and managing accurate large tissue banks that could encompass all physiopathological situations of interest.

Name: Antonio Vidal-Puig

Title: The need for a focused, coordinated European Research Effort to stop the current obesity Epidemic

Obesity, defined as the excessive accumulation of fat, is in fact an important problem but still is not perceived as devastating as cancer or cardiovascular problems. Indeed it seems a little extreme to consider obesity as the "most important health problem" or as the "central component of the metabolic syndrome". Obviously, it is important to increase the awareness about the obesity problem among the public and politicians, but we need to be careful not to inflate the facts since we risk trivializing the subject. Indeed the data currently available about the obesity epidemic are significant enough not to need overstatements. Probably it would be a better strategy to emphasize the fact that decreasing/preventing obesity will decrease the prevalence of very incapacitating and expensive complications, saving lives and money.

Prevention and treatment

Surgical methods to treat obesity are becoming more and more accepted. In fact probably this method is the only one, which has been effective in long term body weight reduction. This is acknowledged in a recent NICE document on bariatric surgery. The experience accumulated with bariatric surgery is now even more interesting after recent research indicating that the intestinal track may be an important contributor to energy homeostasis through secretion of peptides and phospholipids that modulate food intake.

The search for obesity genes

The genetic basis of obesity may not be necessary for treatment at the present moment, since we do not have selective target therapeutic alternatives. However this information may still be useful for counseling and prevention among individuals more predisposed to develop obesity. Early awareness/intervention in these individuals may help to prevent/control the development of obesity and/or its complications, through early behavior modification or pharmacological intervention not only directed to prevent the development of obesity but also its associated complications.

The search for obesity genes

The identification of monogenic forms of obesity may appear at first sight meaningless to identify the mechanisms of more common forms of obesity. However these should be considered as key experiments to unravel the mechanisms controlling energy homeostasis.

There is evidence that common forms of human obesity are polygenic, but also it is evident that the epidemic of obesity has emerged in the last 20 years. Thus an important environmental component must exist. The apparent lack of success of genetic studies probably is due to "lack of power" of the present strategies to identify meaningful but subtle associations. In my opinion it would be important to develop powerful IT technologies capable of analysing simultaneously the interaction between gene variants and environment factors in large populations.

Mouse models are key tools to study the effects of specific genetic modifications *in vivo*. Mouse models may also be used to study polygenic forms of obesity, although this will be certainly more complex and expensive. Obviously the study of diet induced obese mouse models and the influence of specific genetic backgrounds may provide useful information to be translated to human research.

An important limitation would be to have access to IT support to analyse and integrate all the information generated through all these complementary approaches. However, after the genome project, these tools exist, thus is just a problem of having access to them.

Thermogenesis

It has been difficult to identify differences in energy expenditure between obese and lean individuals. However this should not discard that alterations in energy expenditure may be a pathogenic factor for the development of obesity. Indeed it is possible that these studies would shed more light if performed in pre obese individuals. If these individuals could be identified at early stages then changes in energy expenditure may be observed. Even if alterations in energy expenditure were not identified, there is evidence that increasing energy expenditure (e.g increasing mitochondrial uncoupling) may still be a good strategy to lose weight. The challenge is how to increase energy expenditure safely.

Drug treatment

Present treatments have short-term success or undesirable effects, in part due to compensatory homeostatic responses to maintain steady state. Ideally, research should be directed to identify non-redundant systems not susceptible of compensation. To identify these systems it is key to have *in vivo* models with specific targeted genetic modifications. These models allow the study of the effects of these specific genetic modifications but also allow the identification of potential compensatory systems. These alternative systems may be of therapeutic interest. Once key non-redundant systems have been identified and characterized it is likely that obesity may be treated with a multi drug approach targeted to these systems in a similar way as hypertension is treated with drugs directed to multiple targets.

Lifestyle intervention

It is becoming obvious that lifestyle changes (e.g. exercise) have a moderate effect on body weight control probably due to compensatory homeostatic mechanisms. However these changes in life style should be encouraged since they may be extremely

beneficial preventing/delaying the complications of obesity (e.g atherosclerosis, diabetes).

Surgery

There is evidence that morbid obese patients treated with surgery lose weight. It is unclear whether losing weight using this approach is going to be as beneficial as expected. In the absence of better therapeutic options probably this should be considered as the best/unique therapeutic option for morbid obese individuals that otherwise have/will have serious health problems.

Comments to Dominique Langin Paper

My point of view basically agrees with the content of this paper.

Other comments/thoughts

1. Obesity is the excessive accumulation of fat resulting from a positive unbalance in energy homeostasis (EH). Thus obesity may be considered as the failure of the mechanisms controlling EH to maintain normal body weight. It has been suggested that the main priority of the EH system is to survive situations of energy deprivation. However, this system does not seem to handle situations of energy surplus very efficiently. The regulation of EH involves processes that take place in the brain, adipose tissue, gastro intestinal track, liver, muscle etc... The regulation of EH in these organs seems to be a regulated and integrated process at the level of the whole organism. Some of these mechanisms of EH regulation seem to be redundant (e.g NPY) while others are non-redundant (e.g. leptin).

Thus it could be argued that understand and treat obesity it is important to consider the whole organism energy homeostasis and not just the accumulation of fat. Intervention directed to one of the components of the EH system are likely to be counterbalanced by the other components of the system in an attempt to maintain the steady state

Probably the main challenges to address the obesity problem are: a) how to study obesity as a global problem of energy homeostasis and, b) how to integrate and coordinate efficiently the information generated through multiple approaches that generally represent partial pictures of EH system.

2. We all agree that the obesity problem requires urgent action. The problem is to determine the order of priorities. Society and politicians rank these problems in the following order:
 - a. To treat the complications of obesity (e.g. diabetes, cardiovascular etc).
 - b. To prevent the complications of obesity through weight reduction programs.
 - c. To prevent the development of obesity.
3. However, It is clear that to effectively control the obesity epidemic these three problems would require to be addressed simultaneously. Furthermore failure to develop strategies to effectively reduce body weight will generate another subsequent uncontrolled epidemic of complications. Thus in the long term it would be more efficient and cost effective to prevent and treat obesity than its

complications.

4. Thus some of the main problems/areas of research in obesity should include:
 - a. **To develop efficient tools to lose and maintain weight loss:** It is key to characterize the mechanisms controlling energy homeostasis with the aim of identifying non-redundant pathways susceptible of drug intervention.
 - b. **To effectively prevent obesity:** it is necessary to identify individuals at risk of developing obesity and/or prone to develop obesity associated complications to implement early intervention. Given the polygenic/heterogenic character of this syndrome and the relevance of the environmental factors on its development, genetic analysis should include the study of gene-gene, and gene environment interactions.
 - c. **To decrease the impact of obesity related complication;** it is important to define how excessive accumulation of fat in specific adipose tissue depots or ectopically promotes insulin resistance, defects in insulin secretion, atherosclerosis, hypertension etc etc. In this context the exploration of unified pathogenic mechanism such as lipotoxicity, glucotoxicity, or inappropriate inflammatory responses should be considered as a priority.
5. To study the obesity problem it is required an integrated multi approach strategy. It is anticipated that recent technological developments will have an important impact in the way the obesity problem is approached. Some of the tools/approaches available include:
 - a. Cell biology
 - b. In vivo physiological studies in animal models
 - c. In vivo physiological studies in humans
 - d. Genomics
 - e. Proteomics
 - f. In vivo imaging
 - g. Genetics
 - h. Epidemiology
 - i. Neurosciences
 - j. Psychology
 - k. Availability of High throughput technologies
 - l. IT support
 - m. Incorporation of new emerging technologies
 - n. Mathematic models of energy homeostasis
 - o. Physics

p. etcetcetc

Some of the challenges that this multi approach strategy poses is how to have access to these technologies, how to integrate the work of experts in these areas and how to utilize efficiently all the information generated by this complex network.

"Improving Health through Diet"

Name: Abdul G. Dulloo

Title: Maladaptive Thermogenesis in Obesity : Molecular-Physiology and Dietary management Research

The 'catch-up fat' phenomenon:

a common denominator in the link between weight perturbations across ages (fetal, neonatal, childhood, adulthood) and high risks for obesity and diseases later in life

In the position papers on 'Early nutrition and Programming' and 'Is there early metabolic imprinting of later obesity risk', Dr Ozanne and Dr Koletzko have both underlined the evidence suggesting that metabolic perturbations of regulatory systems established during fetal and neonatal periods contribute to the development of obesity, type 2 diabetes and cardiovascular diseases later in life. I would like to add that:

- i. high risks for obesity and chronic diseases have also been reported when growth retardation earlier in life occurred during childhood (16-19),
- ii. those people with the highest susceptibility for abdominal obesity, impaired glucose tolerance, type 2 diabetes, and cardiovascular diseases later in life are those who had low birth weight or who were stunted during infancy or childhood, but who subsequently showed **catch-up growth** (12-15), and
- iii. furthermore, high cardiovascular morbidity and mortality have also been reported in men and women who in young adulthood experienced weight fluctuations (involving the **recovery of body weight** after substantial weight loss due to disease, famine or due to voluntary slimming/'yoyo' dieting) (3-7).

The question that arises therefore is why the phases of catch-up growth or weight recovery (in adults) seem, *a priori*, to be critical periods which render individuals particularly prone to the development of high risks for obesity and diseases? Current hypotheses center upon notion that malnutrition, particularly when it occurs during critical periods of growth and development, can lead to lasting alterations in the structure or function of tissues and body systems. Such malnutrition-induced 'programming' or 'imprinting', although adaptive during the period of limited supply of nutrients, are thought to contribute to increased risks for diseases during improved nutrition later in life (20). While the early mechanisms that underlie such predispositions remain obscure, a **common denominator** in all these situations of large weight fluctuations is that body fat is recovered at a disproportionately faster rate than that of lean tissue, thereby underscoring a potentially pivotal link between processes that lead to accelerated fat recovery (or catch-up fat) and higher cardiovascular risks.

Physiology and pathophysiology of 'catch-up fat':

What then do we know about the processes that regulate catch-up fat after growth retardation/weight loss? An exaggerated increase in energy intake (*compensatory hyperphagia*), particularly during catch-up growth/weight recovery on energy-dense diets (high in fat, refined carbohydrates and salt), is often implicated in the development of excess adiposity, insulin resistance, hyperinsulinaemia and an overactive sympathetic nervous system. However, a disproportionately high rate of fat deposition during catch-up growth/weight recovery still occurs in the absence of hyperphagia (32, 35, 36, 40, 45), which hence underscores **an elevated efficiency of body fat recovery** as a fundamental 'adaptive' physiological reaction to growth retardation/weight loss. There is indeed converging evidence from experimental studies of prolonged starvation and refeeding in humans (34, 49, 50) and in animals (51-54) that such an elevated efficiency underlying accelerated fat recovery is a phenomenon that occurs at all ages, and that it is a carryover effect of the diminished thermogenesis that occurred in the preceding period of food deprivation. These findings are in support of the existence of an autoregulatory control system which participates in the regulation of catch-up growth/weight recovery by sustained suppression of thermogenesis, and that the energy thus conserved is directed specifically for 'catch-up fat' rather than for catch-up of the lean tissue. It is viewed as a control system that operates as a feedback loop between depletion or delayed expansion of the fat stores and suppressed thermogenesis – and has been referred to as *an adipose-specific control of thermogenesis* (48) Given these close associations between catch-up growth/weight recovery, a high metabolic efficiency underlying catch-up fat and the development of obesity and diseases later in life, the possibility therefore arises that a sustained reduction in energy expenditure *per se* (due to diminished thermogenesis in certain organs/tissues) – for the purpose of

enhancing the efficiency of fat recovery – is also involved in the pathogenesis of these chronic metabolic diseases. Recent studies in animal models of ‘catch-up fat’ support this contention (see ref below).

Areas for Research:

Molecular-physiology - In the present state of knowledge, the components of this control system - *the sensor* of the state of body fat depletion/repletion, *adipose-derived signals* that lead to suppressed thermogenesis, *effector sites* and *molecular mechanisms* involved - remain within a black box of autoregulation of catch-up fat. Elucidation of the components of the *adipose-specific* control of thermogenesis, how they are modulated by diets high in fat, whether they are 'hypersensitized' with repeated cycles of weight loss and weight recovery, by foetal or neonatal programming, and their relevance to ‘adiposity rebound’ in children, are crucial steps towards defining the molecular-physiological pathways from weight fluctuations to obesity and chronic diseases.

Nutritional management - There is also a need for research into dietary approaches – via specific macro- or micronutrient compositions, supplements or functional foods - that can override the ‘maladaptive’ suppression of thermogenesis that characterises the catch-up fat phenomenon during catch-up growth or weight recovery during adulthood.

References in brackets refer to those in ‘Reference’ section of following review article: Dulloo, Jacquet & Montani: ‘Pathways from weight fluctuations to metabolic diseases: focus on maladaptive thermogenesis during catch-up fat’ *Int. J. Obes.* (2002) **26**, Suppl 2, S46-S57.

Name: Marinos Elia

Title: Brief comments on documents submitted for ERA workshop

General

There appears to be a need to integrate the research areas into a European strategy, in the light of EEU agenda, which includes wealth creation, consumer interests, and competition with USA and Japan.

The World Health Organisation has recently provided guidelines for desirable BMI ranges (and underweight and overweight/obesity) for Asians and Caucasians. The values for Asians are lower than for Caucasians. It is also becoming clear that there are different BMI-%fat relationships between Asians in different countries, as well as Asians within the same country (e.g. north and south India, and north and south China), and that these are related to risk of premature morbidity and mortality. A concerted European action should consider potential body composition differences amongst various countries, and especially whether at the same BMI, there is more % body fat and more central body fat distribution in some European countries than others (analogous to the Asian/Caucasian situation). Such considerations may also help explain other risk factors that are linked to morbidity/mortality from common diseases, and the extent to which manipulations in early or later life may be modulated through changes in body composition. A co-ordinated multinational approach linked to the other initiatives discussed in the position documents should be considered. These activities could be linked to the International Body Composition Group, and the recent plans by the international committee to establish a new reference man, or series of reference men and women. This could involve a 'reference' European man and woman.

Perspectives: molecular genetic research in human obesity (J Hebebrand)

This is a useful summary of the potential value and limitations of molecular genetic research in humans. One of important limitations is that the individual genes have been found to account for only a very small proportion of obesity. Therefore, it is necessary to consider strategies for combining genetic and other information to extend the potential value of the genetic approach. Behavioural and lifestyle factors are undoubtedly important in the aetiology of obesity, but the extent to which these are linked to genetic factors is unclear, since the genetic basis of behaviour is less well studied than that of physical traits. If gene-behaviour interactions are to form the basis of a major European research strategy relevant to obesity, it is important to consider in much more detail the type of behavioural characteristics and candidate genes, together with clinical/public health approaches to management.

Is there early metabolic imprinting of later obesity risk? (B Koletzko)

The reduced risk of obesity in children who are breast fed suggests a potentially important form of intervention, which is consistent with recommendations by international to breast feed in preference to bottle feeding. The consistent findings from at least three separate studies showing reduced risk of obesity in breast fed infants after controlling for other variables, adds strength to the proposition. However, it still does not prove that it is the composition of breast milk that is responsible for the observations. For example, it is possible that different associated factors, such as bonding during breast feeding (or other life-style factors linked to breast feeding) are at least partly responsible the relationships across studies.

The CHOPIN study is an important prospective intervention study and the results are eagerly awaited. The putative long-term metabolic programming effects of protein in animal studies (glucose intolerance, hypertension, ? obesity) relate to low protein feeding during foetal and post-natal development. In contrast, the CHOPIN study examines the effect of increasing the protein intake above the average level. An integration of these two sets of observations would be welcomed.

The effect of breast feeding in reducing the obesity 'tail' rather than shifting the population mean suggests a specific effect of breast feeding in particular groups of individuals. Strategies that aim to shift the population mean, even if this is to a small extent, could prove to be a more powerful way of dealing with the public health problem of obesity than shifting a proportion of the obesity 'tail' to a larger extent.

The use of the same centile cut-off values for defining overweight and obesity raises potential problems when comparing obesity/overweight indifferent European countries, which have different BMI distributions (see general note above - body composition and fat distribution).

Genomics and biotech research area

The three papers listed below are addressed conjointly by H-G Joost, S Ozanne and D Langin

A wide range of interventions is proposed, including those that aim to increase birth

weight and modulate catch-up growth. However, it is possible that these variables are markers of other biological processes, which are directly responsible for programming. If this is the case the some manipulations to increase birth weight or modulate catch-up growth may not achieve the desired effects. There is a need to understand the mechanisms of foetal and early origins of adult disease, through genetic and nutritional interventions in both humans and animals. An integrated approach taking into account the research directions suggested by the three authors, including polygenic animal and human studies, foetal origins of adult disease, and potential mechanisms linking these areas, would be worth considering further.

Name: John Blundell and **Clare Lawton**

Title: Measuring Appetite Control Risk Factors (arising from biology, exploited by the environment)

The position papers have drawn attention to genetic factors, fetal programming or early nutritional experiences to account for a disposition to gain weight and to become obese. One important feature of these processes is that they may all work (at least in part) through appetite control mechanisms (e.g. disturbing satiety signaling, sensitizing appetite drive, impairing the capacity for compensation or biasing food preferences). Given the extent to which the (obesigenic) environment exploits behavioural dispositions for raising energy intake (Blundell and Cooling, 2000), the research effort of the Leeds group has been directed towards identifying the most significant measurable risk factors that act, primarily through the control of appetite, to bring about a positive energy balance. The strength and number of opportunities provided by the environment to over-consume (via technological advances in food science, food delivery systems, and marketing) suggests that a major route to weight gain is through the appetite control system. The Leeds position is based on the existence of individual differences in the susceptibility to over-consumption (via different mechanisms) that can be identified through appropriate screening and characterization. These susceptible patterns (which may be clusters of factors) can co-exist with metabolic factors that either protect against, or augment, the tendency to gain weight.

Homeostasis and Hedonics

Over the years, 2 rather separate systems influencing appetite control have been described. The most researched system involves the principle of homeostatic (regulatory) control and contains tonic and episodic signaling systems. Signals arising from basal metabolism and adipose tissue constitute largely tonic control, whereas signals arising from food consumption comprise an episodic signaling system whose components vary in strength and duration. This entire system involves a matrix of neuropeptides including leptin, NPY, Agrp, CART, POMC, MC-4R etc (Berthoud, 2002). A largely separate system is based on hedonic aspects food consumption and exerts its effect on appetite control via modulation of the perceived pleasantness or reward value of food. This system operates via neurotransmitters such as glutamates, dopamine, endogenous opioids, and endocannabinoids. Although the systems are structurally relatively independent, they can interact. For example, the hedonic value of food influences satiation (meal size) and CB-1R interact with leptin levels. The existence of these 2 systems suggests different ways in which appetite control can be influenced to promote overconsumption. It also suggests features of the appetite control system (behavioural traits, psychological states, sensory responses) that can be identified and characterized. The programming of appetite control (by genetic, metabolic, early environmental factors) may involve a defect in satiation (leading to enlarged eating

episodes), a preference for fatty tasting foods (Mela and Sacchetti 1986), preferential consumption of high energy (high fat) food products (Westerterp et al, 1997), a readiness to eat opportunistically (respond to environmental triggers), tendency for altered pleasure from eating, disposition to give way to binges. All of these traits or dispositions can be objectively measured via behavioural tests or validated questionnaire methods. The human pleasure response can be operationalized and treated as a scientific variable.

Regulatory breakdown: failure to compensate

Genetic influence or early programming could disturb appetite control by impairing the regulatory processes that normally work towards the maintenance of energy balance. Together with the Human Nutrition Group at the Rowett Research Institute, we are investigating the capacity of people to compensate for imposed interventions in energy intake (over or under consumption) or energy expenditure (increased physical activity or increased sedentariness). Compensation for energy deficits is stronger than for an energy surplus (or positive energy balance). However, there is marked individual variability; there exist good and poor compensators. This feature can account, in part, for people who are either susceptible to weight gain or who fail to benefit from physical activity. The measurement of the capacity to demonstrate adjustment (compensation) to a perturbation of energy balance is a measurable variable to diagnose impaired appetite control.

Heritable Components of Appetite Control

There is now considerable evidence to indicate the heritability of specific components of appetite control (i.e. – capacity for these components to be transmitted across generations), presumptive physiological mechanism associated with a behavioural trait, or a detectable relation to weight gain in otherwise identical individuals (e.g. MZ twin pairs). For example, the control of meal size (de Castro, 1993), hedonic response to foods via DA-2R density (Wang et al, 2001), trait of ‘disinhibition’ (heritability estimate of 0.40 among Amish – Steinle et al, 2002), high fat preference (heritability of 0.60 based on concordance of MZ, DZ twins, Platte – personal communication), selection and consumption of high fat foods. There is a lesson here from the dramatic example of leptin deficiency. This single gene defect leads to obesity through a behavioural mechanism i.e. unchecked hyperphagia. Other research on weight loss indicates a strong association between hunger and leptin levels.

Characterizing Appetite Control through Behavioural Phenotypes

The Leeds approach to identifying key dispositions in appetite control mechanisms is initially to characterize individuals with specific behavioural traits (later these can be linked to anthropomorphic indices i.e. leanness and fatness). Habitual consumption of high fat or low fat foods has emerged as a productive research focus. This trait can be objectively measured (although with some difficulty), appears to have a heritable component and is associated with metabolic adaptations (RMR, RQ, night time HR) in those HF phenotypes who remain lean. In the HF phenotypes who become obese the behaviour is characterized by high TFEQ ‘disinhibition’ (tendency to eat opportunistically), heightened pleasure response to fat foods (hedonic component), demonstrated preference for high fat foods, and high consumption in intake tests (Le Noury et al., 2002). All of these features (traits, risk factors) can be measured and have putative links with physiological mechanisms or genetic markers. The objective measurement of appetite expression can be achieved and can be used to establish

linkage in genetic screening or to assess the effects of fetal conditions or early environmental conditions (e.g. breast feeding).

Linking Biology and Environment: joined-up thinking

The establishment of an inventory of objective traits that reflect the programming of appetite control can be used to confirm biologically based dispositions, and also to devise strategies for improving health through dietary/behavioural management. Strategies will probably need to be based not on a 'one size fits all' philosophy, but on a series of programmes matched to particular clusters of appetite risk factors. At this stage it would be very useful to develop a consistent and uniform package of appetite assessments to facilitate comparisons between the research outputs of research groups working with different strategies (genetic, fetal, early environment etc). We propose a consensus conference on this issue to develop a work-package of instruments and methodologies for measuring appetite control and to co-ordinate a European approach.

Name: Katherine Macé

Title: Response to position papers on the "Early Nutrition and Programming" session

The following response will be focused on the potential protective effects of breast feeding on childhood obesity with firstly a short discussion and secondly some potential axes of future research in this field

Discussion

The most comprehensive review on longitudinal studies in industrialized countries indicated that the risk factors for later obesity include parental fatness, social factors, birth weight, physical activity, dietary and behavioral factors .

The debate on the protective effects of breastfeeding vs infant formula on childhood obesity is still open due to conflicting epidemiological results and lack of intervention studies, even in animal models.

Observation studies show that weight gain and adiposity in infancy are linked to energy intake rather than feeding practice . Interestingly, Fomon followed 470 children until 8 years. At 4 months bottle-fed babies were longer and heavier than breastfed babies and their weight/height ratio was greater. There was a highly significant correlation between fatness at 4 months and at 8 years. However there was no correlation between the feeding pattern and fatness at 8 years .

Today, specific components of human milk that could reduce the risk of obesity in breast-fed children have not been identified. In terms of nutrient intake, the major difference between human milk and infant formula is the protein content and protein intake of breast-fed infants has been shown to be lower than in populations of formula-fed infants . Nevertheless, two groups have found opposite results in testing the hypothesis that high proteins early in life represents a predisposition for later obesity. One study done by Rolland-Cachera and Bellisle's group indicated that a high protein intake at the age of 2 years increases body fatness at 8 years of age, via an early adiposity rebound (AR). According to this report protein at age of 2 years was the only nutrient intake associated with fatness development pattern. Nevertheless, protein intake appears as a weak determinant since it explains only less than 5% of the total variation. The study done by Dorosty and Reilly did not support the hypothesis that early AR is promoted by high-protein intake. None of the dietary variables tested were

significantly associated with timing of the AR, and timing of AR was not associated with socioeconomic status. Parental obesity was associated with an earlier AR .

Childhood obesity is a multifactorial problem with a wide range of environmental and behavioral factors associated with genetic predispositions. Breastfeeding includes both environmental and behavioral factors, which could have some preventive effects on the development of obesity in later life. Nevertheless, today there is no clear-cut evidence to confirm or invalidate this hypothesis. Because childhood obesity prevention is today a major public health priority, the role of nutrition on early metabolic programming of later obesity risks needs to be rapidly further investigated

Potential future research

Long-term well-controlled studies in infants should obviously clarify the debate on the protective effects of breastfeeding. Nevertheless, this type of study is difficult to put in place and exclusivity of breastfeeding and its duration should be tightly controlled as well as other known risk factors including parental fatness, social factors, and birth weight.

The influence of breastfeeding on the prevention of obesity later in life should be further evaluated in animal models. Non-human primates could be used for well-controlled studies as previously done for assessing the role of energy intake during the suckling period on adiposity later in life . The artificial rearing technique allows today nutritional investigations to be conducted in rat pups during the suckling period . Nevertheless, relevant animal models need to be identified since for instance great interspecies differences exist in the time course of the development of adipose tissue. In most species, excepted human, body fat content at birth is very low and white adipose tissue is barely detectable .

This research field also requires the development of reliable biomarkers able to predict early in life an increased susceptibility to weight gain later on. In rats, hyperinsulinemia, and probably altered IGF-2 expression in pancreatic cells, developed early in life are associated to obesity during adulthood . Leptin which plays an important role in modulating adaptation to energy regulation and utilization and which is positively related to fat mass is another interesting candidate. Nevertheless, the hypothesis that early programming of leptin concentrations may be one mechanism by which perinatal nutrition influences later obesity needs to be demonstrated. Finally, in order to speed-up the discovery of potential biomarkers, "omic" (genomic, proteomic, metabolomic) assessments in this research field should be rapidly implemented to build integrated databases of gene/protein expression as well as metabolite concentrations across human and research animal populations.

References

Name: David J Mela

Title: Increasing the Impact of European Obesity Research

Obesity presents a present and rapidly growing threat to the well-being of individuals and health care systems across Europe. To increase the impact of obesity research in the European Research Area, priority should go to topics with clearly **actionable results**, which can deliver timely and meaningful health and economic benefits for our citizens, economies and industry.

Research is likely to be "actionable" when:

1. The additional resource and time that would be required apply the results, and *feasibility* are quantified and acceptable
2. It is absolutely clear *how* and *by whom* the research would be applied, and the likely timeframe for this.
3. There is realistic probability of success and also implementation if successful, and implementation would have *meaningful* impact

These criteria, and the urgent need to address obesity as a health care problem, should guide current decisions on priorities for obesity research.

Obesity differs from traditional diseases by its strong behavioural and environmental determinants. It is not limited to a small, genetically "at risk" group of people, and rarely linked to any remarkable physiological determining feature. It is therefore disappointing that many proposed topics in obesity are still based on the notion of a "defect", and the position papers for this meeting are so heavily dominated by physiological and biochemical reductionism. In many cases, it is not clear when, how or by whom results would ever be realistically implemented. For others, pharmaceutical companies are the primary or only possible beneficiary of the research. We must ask if this is really the best route toward meaningfully impacting upon today's obesity crisis. Specifically, we propose that areas such as molecular genetics and drug target development should have only limited and *strategically focused* support from a European obesity research programme. These are better funded through other ERA or national science programmes, or in partnership with by relevant industries.

To slow the obesity epidemic in Europe, individual and environmental interventions offer the greatest and most rapid uptake and impact. Yet research on assessment and implementation of such interventions has had relatively little support, and hardly features in many of the position papers. Longer-term benefits may come by searching for physiological or genetic "fingerprints", but the immediate need is for understanding and testing of effective and feasible ways to alter the environment (including food) and encourage appropriate individual lifestyles and behaviours. These are areas where European researchers can have tremendous scientific and policy impact.

We therefore strongly recommend a bold and significant strategic shift toward a more holistic and actionable obesity research programme centred on social/behavioural, nutritional and public-health oriented research, with attention primarily directed toward:

1. Epidemiology and tracking of obesity and co-morbidities

- Historical patterns and future trends in Europe
- Harmonization of survey and monitoring systems (including health care and economic costs)

1. Identification and quantification of causal factors and intervention targets

- Retrospective analyses of exposure, and identification of potential causal factors to be monitored in prospective epidemiological studies
- Lifestyle, behaviour and environmental setting of individuals who are obesity "resistant".

- Detection of target groups and life stages associated with heightened risk of weight gain
- Quantitative experimental intervention and simulation studies to assess the implementation, feasibility and potential impact of population-level measures (e.g., environment, public health, food composition and marketing) as targets for prevention and treatment, with emphasis on prospective weight gain and development of obesity. To include:
 - Impact of specific systematic changes in behaviour or environment
 - Scale-up studies and analyses of "minimal" interventions (e.g., population-wide effects of relatively small but widespread individual changes in e.g. food composition or activity)
 - Feasibility and economic analyses
 - Potential policy framework and legal implications

1. Intervention and prevention

- Optimizing recommendations for physical activity patterns for weight control
- Blood glucose regulation, insulin sensitivity and progression of diabetes Type II in relation to overweight and obesity, including dietary factors, and glycaemic response to foods and its role in weight control and health.
- Dietary and lifestyle approaches to prevention of diseases of obesity, especially insulin resistance and diabetes, including food composition, form, and eating patterns
- Evaluation of individual and family prevention and treatment interventions for overweight and obesity, and development and dissemination of best practice guidelines, including:
 - Prevention strategies for families, children and teenagers (including "critical periods")
 - Cost-effectiveness analyses
 - Initiating behaviour change and maintaining compliance
 - Influences of foods and dietary and activity patterns on:
 - Metabolic signalling and substrate utilization
 - Gastrointestinal responses and adaptation relating to appetite control
 - Behavioural (intake) responses to foods
 - Targets and agents for (functional) foods for weight control, including:
 - Test methodology and claim substantiation criteria
 - Improving the satiety value and appetite control effects of foods

- Benefits for substrate oxidation, thermogenesis and nutrient partitioning

1. Consumer understanding and communication

- Optimal communication and advertising approaches to promote effective weight control behaviours, including daily physical activity
- Communication to consumer and health professionals of obesity as a health risk
- Consumer understanding, and behavioural and marketing models for commercial promotion of activity and "healthy" eating (including "functional" foods and health-related claims)
- Effective education at schools to children on healthy diets/lifestyle
- Food marketing, especially to children, and health communication strategies
- Informative, consumer-friendly nutrition labelling schemes

Name: Len Storlien

Title: Response to the "Improving health through diet" research area

In the absence of a position paper, the following are areas of perceived greatest research need in the area of "Improving Health Through Diet" representing, very briefly, a personal perspective only.

First, the contribution of diet, and dietary change, to the epidemic of obesity and the related co-morbidities of the Metabolic Syndrome is not clear. It has been difficult to document major increases in dietary fat (either as total calories or as a percentage of calories) in the time period of the greatest rate of increase in obesity. Equally, while intake of simple sugars, in particular fructose, has risen sharply in the American diet the direct linkage of this observation to increase in obesity prevalence has not been made. Clearly, one limiting factor in establishing causality is the great limitation of dietary intake methodology. Improved methodology, perforce via novel biomarkers, is much needed.

Long-term (at least 6 months, preferably 1 to 2 years) intervention studies are a very high priority. The emphasis on the outcomes measures of the proposed work should be not only on weight per se, but also on measures which reflect "metabolic fitness". Thus reduced body fatness, fat redistribution away from visceral fat, improvement of insulin action and glycemic control, amelioration of dyslipidemias and of inflammatory markers must all be a part of the outcomes package – as must genotyping to allow discrimination of genetic factors which associate with specific outcomes profiles.

From a good deal of experience in long-term dietary intervention studies in "free-range" humans, changes dietary macronutrient proportion are difficult to sustain. In contrast, modulation of the subtype profiles within each macronutrient category, is achievable and sustainable. Enough evidence has accumulated to argue that fatty acid, carbohydrate, protein and fibre subtype profile have a profound influence on body weight, insulin action and blood lipid parameters. Much of this work is done in experimental animals and a very high priority is to establish unequivocally, the same data in humans. A concerted effort by a consortium of laboratories to establish what is known, likely and suspected based on a thorough analysis of the existing literature must

be the starting point. Then the intervention studies must be carefully planned, and of sufficient size and duration, to provide the sort of data which can be used to drive change via government regulatory bodies and the food industry. It is to be recognised that the intervention studies must not fail to be supported, as they have in the past, because they fall in the gap between tightly controlled "hard experimental" and population science.

While the role of dietary intervention in obesity treatment is of prime importance, simple dietary intervention studies which have emphasised just realistic lowering of caloric, or fat intake, have achieved only very modest success and even that limited weight lost is almost invariably regained. Equally the greater weight loss provoked by VLCD (often > 10 kg over periods of 12-16 weeks) is not seen to be maintained during the subsequent "maintenance" period. Clearly, an understanding of the altered metabolism of the "weight-reduced" state is critical as it will inform the directions for research into better dietary formulations to better maintain the weight-reduced state. Here, much greater knowledge of diet-gene interactions is necessary. It is clear, for example, that lipids (both fatty acids and cholesterol) are potent regulators of gene expression with potent differential effects of fatty acid subtypes on expression of multiple genes of endogenous lipogenesis and fatty acid oxidation via differential induction or suppression of cellular master control elements such as nuclear hormone receptors and sterol regulatory binding proteins. In this context the study of Luan et al (Diabetes 2001) is instructive as they have demonstrated that the relation of various alleles of the PPAR γ 2 gene to BMI is highly dependent on the P/S ratio of the diet. The common Pro12Pro allele is linked to low BMI only if the dietary P/S ratio is low; if the P/S ratio is high then the relationship is exactly the inverse. Studies of this nature must be strongly encouraged.

In the etiology of obesity, physical inactivity is likely to play at least as strong a role as diet and understanding the interactions between intake, the profile of that intake (types of food, drivers of intake, situational cues, etc. – particularly in children), and sedentary behaviors is critical. For example, with less physical activity we know that the capacity of skeletal muscle for fat utilisation is diminished. Is this enough to tip the fat balance to positive over the long-term or is the interplay between physical inactivity, diet, and development of obesity more complex and multifactorial? – critical research questions.

Name: Aileen Robertson

Title: First Food & Nutrition Action Plan for the WHO European Region 2000-2005

Access to a safe and healthy variety of food, as a fundamental human right, was stressed by the International Conference on Nutrition in 1992 and by the World Food Summit in 1996. A supply of nutritious and safe food is a prerequisite for health protection and promotion. In spite of commitments expressed and efforts made at national and international levels, there is still a need for policies which reduce the burden of food-related ill health and its cost to society and health services.

Low breastfeeding rates and poor weaning practices result in malnutrition and disorders such as growth retardation, poor cognitive development, and digestive and respiratory infections in young children. Iodine deficiency disorders affect around 16% of the European population and are a major cause of mental retardation. Iron deficiency anaemia affects millions of people and impairs cognitive development in children and, during pregnancy, increases the risk to women.

The prevalence of obesity is up to 20–30% in adults, with escalating rates in children, increasing the risk of cardiovascular diseases, certain cancers and diabetes. Obesity is estimated to cost some health services about 7% of their total health care budget. Around one third of cardiovascular disease, the first cause of death in the Region, is related to unbalanced nutrition, and 30–40% of cancers could be prevented through better diet.

In countries of the European Union, a preliminary analysis from the Swedish Institute of Public Health suggests that 4.5% of disability-adjusted life-years (DALYs) are lost due to poor nutrition, with an additional 3.7% and 1.4% due to obesity and physical inactivity. The total percentage of DALYs lost related to poor nutrition and physical inactivity is therefore 9.6%, compared with 9% due to smoking. New figures in the 2002 World Health Report on the Global Burden of Disease support this.

The Food & Nutrition Action Plan for the European Region stresses the need to develop food and nutrition policies which protect and promote health and reduce the burden of food-related disease, while contributing to socioeconomic development and a sustainable environment. It insists on the complementary roles played by different sectors in the formulation and implementation of such policies. It provides a framework within which Member States can begin to address the issue. The framework consists of three interrelated strategies:

- A food safety strategy, highlighting the need to prevent contamination, both chemical and biological, at all stages of the food chain. The potential impact of unsafe food on human health is of great concern, and new food safety systems which take a "farm to fork" perspective are being developed.
- A nutrition strategy geared to ensure optimal health, especially in low-income groups and during critical periods throughout life, such as infancy, childhood, pregnancy and lactation, and older age.
- A sustainable food supply (food security) strategy to ensure enough food of good quality, while helping to stimulate rural economies and to promote the social and environmental aspects of sustainable development.

The action plan was endorsed by all ministries of health in the European Region (51) for the period 2000–2005, with approaches and activities to support Member States to develop, implement and evaluate their food and nutrition policies.

The need for coordination between sectors and organizations will increase as ethics and human rights, in addition to science and economics, play a greater role in decision-making. Countries should consider which mechanisms are needed to facilitate better coordination between sectors and ensure that health and environmental concerns are considered when food and nutrition policies are made.

The nutrition strategy

Nutritional challenges vary as we progress through the life cycle. Good nutrition during the first few years pays dividends throughout life. This starts with maternal nutrition, because of its importance to the foetus and the evidence that nutritionally-related low birth weight raises the risk of cardiovascular disease in later life. The failure of pregnant women to obtain a safe and healthy variety of food has long-term social and economic consequences. The WHO Regional Office, with UNICEF, has developed training materials to help health professionals improve the health of women and their children

with safe food and good nutrition.

Analyses demonstrate that exclusive breastfeeding and the introduction of safe and adequate complementary foods from the age of about six months while breastfeeding continues, can reduce the short- and long-term burden of ill health. The Innocenti Declaration on the protection, promotion and support of breastfeeding was adopted as a basis for policy by the World Health Assembly in 1991, and the WHO Regional Office monitors its implementation in Member States. More recently the Office, together with UNICEF and with support from the governments of the Netherlands and the United Kingdom, has published new feeding guidelines for infants and young children.

In adolescence, the health impact of nutrition is pronounced. During their periods of rapid growth, adolescents have increased energy needs. Many of them, especially those in low-income groups, choose relatively cheap sources of energy, such as large amounts of fat and sugar, potentially leading to micronutrient deficiency, obesity and dental caries. Increasingly, there is evidence that poor nutrition due to income inequalities results in health disparities. The European Network of Health Promoting Schools, in collaboration with the Regional Office and the EU Commission, has produced a training manual for teachers. In addition, an extensive survey, carried out regularly in almost 30 countries, includes results on adolescents' eating habits and their attitudes towards their body image.

In adulthood, the main challenge is to avoid premature death from cardiovascular diseases and cancers. To prevent these, the dietary guide issued by WHO's countrywide integrated noncommunicable disease intervention (CINDI) programme, recommends "Twelve steps to healthy eating" including eating at least 400 g of vegetables and fruit daily. WHO has also developed guidelines to encourage increased physical activity as part of regular daily living. The aim is to make daily physical activity an easy choice and thus to prevent obesity, as well as reduce the risk of diabetes, heart disease and stroke and promote good health and well-being.

The issue of healthy aging is also of major concern. With decreasing activity levels energy needs are reduced, so the food eaten by older people should be rich in micronutrients in order to compensate for the reduction in food intake. Again, 400 g of vegetables and fruits is the daily intake for older people recommended by WHO. Degeneration of eyesight, lower resistance to infection and other micronutrient-related deficiencies can coexist with obesity, making the health management of older people difficult for professionals.

In addition to commitments made by ministries of health through WHO mechanisms, the EU Council of Ministers recently endorsed an EU Council Resolution on the need for a European Nutrition Action Plan to combat the growing epidemic of obesity in Europe.

"Early Nutrition and Programming"

Name: Gérard Ailhaud

Title: Response to the discussion documents

The selected research themes of this meeting are of interest and the contribution of plenary lecturers is excellent. In my view, the priority should be the prevention for next generations of becoming overweight and obese, i.e. to focus our studies on the early molecular and cellular events occurring during pregnancy and/or lactation imprinting, etc...), and on nutritional aspects in early childhood (theme 3). Generally speaking, if the susceptibility to obesity is determined by genetic factors, we should agree on the critical role of rapidly evolving environmental factors which determine phenotypic expression **at a time** where adipose tissue develops dramatically and where confounding factors (physical inactivity ...) are **not yet** a major issue. Surprising enough, in the position papers, growth and properties of adipose tissue are hardly mentioned as if it were a passive organ. However, it is clear that hyperplastic growth is a nearly « weightless » phenomenon whose consequences on fat mass may appear a few years later.

Importantly, after most studies which have dealt with quantitative aspects of ingested food, it would be **now** appropriate to look at qualitative issues including, whenever possible, retrospective studies in relation to the obesity epidemic. In my view, the critical importance of the food industry in the past and the future can no longer be ignored in our field and requires extensive discussion at this meeting.

Although improving health through diet (theme 2) is also relevant to this industry and is of interest to both clinicians and obese patients, the drug industry will still remain the main contributor to find successful treatments as (too ?) numerous target gene products have been already identified, and more are coming on a monthly basis !!

Regarding theme 1 « Genomics and Biotech », the molecular basis and systemic physiology of chemoreception (taste and olfaction) is a difficult and exciting area which

requires deeper insights. However, on a population basis, if one considers the cultural issues of food habits and the intricate network regulating food intake, which makes its regulation very difficult to tackle, research on thermogenesis should have a high priority.

Name: Jon Arch

Title: Comments on position papers

I don't disagree with any of Dr. Ozanne's questions, but would like to offer some different ideas.

A. There is evidence that increased postnatal weight gain is associated with reduced satiety in SGA infants and that severe calorie restriction during pregnancy leads to hyperphagia in adult life. However, I think it would be a mistake to conclude from this that fetal programming affects appetite and not energy expenditure.

1. Small imbalances in energy balance cause obesity: a 5% error for a 2500kcal/d turnover leads to a gain (or loss) of 6.5kg adipose tissue in a year. We should not expect to be able to detect small errors in energy intake or expenditure.

2. Obese people have a raised metabolic rate and therefore a raised appetite, but this does not mean that raised appetite alone was the cause of their obesity.

3. John Blundell's thin, high fat eaters seem to stay thin by burning off a raised caloric intake.

4. Nancy Rothwell showed that early life exposure to cold could protect against subsequent obesity by promoting thermogenic capacity.

Therefore, I suggest that we should investigate the effect of fetal programming on capacity for thermogenesis as well as appetite control.

B. Low birth weight may not be the cause of susceptibility to obesity but simply a marker of fetal malnutrition. We should consider whether we can reduce susceptibility to obesity irrespective of whether birth weight is raised. My own group has shown that this can be done by giving the mothers leptin. Other treatments of the mother (diet, exercise) or the SGA infant (leptin in milk) might achieve the same goal and have the potential for making a real difference to human health.

C. We need markers (e.g. transcriptomics, metabolomics) that predict susceptibility to obesity if we are to investigate interventions in pregnant humans. Initially work can be conducted in rodents but then markers can be evaluated in humans. For example:

D. Smoking reduces birth weight and there is a recent report that it promotes obesity in offspring. What possible markers of susceptibility to obesity are altered in the small babies of smokers?

Position paper by B. Koletzko et al

It is clearly important to investigate further the role of breast feeding in programming infants to a susceptibility to obesity, through research into Early Nutrition and Programming should not be dominated by this aspect. Since leptin is absorbed from

milk (Locke, Acta Paediatr. 91, 891-896, 2002), I would have liked to have seen its possible role mentioned.

Name: Bernard Beck

Title: Fetal programming and early postnatal dietary experiences: profound influence of type and time distribution of food ingested on subsequent offspring development

As pointed out by many papers, the obesity epidemic is a worldwide phenomenon with a particular intensity in the developed countries. The U.S. situation is a living example of what might rapidly occur in Europe if nothing is undertaken to stop or at least to slow down the expansion of this epidemic.

Our long experience in testing in animals drugs that can diminish energy intake has provided the proofs that this pharmacological approach is useful but does not constitute the ultimate tool to resolve this problem. Indeed, these drug treatments are lifelong treatments to maintain lower body weight and avoid the body weight rebound after cessation. Tolerance to the drugs appears more or less rapidly and these treatments are not devoid of adverse side effects that are in rare cases incompatible with long-term survival of the patients. It is then clear in the mind of all specialized clinicians in this domain that a voluntarily change in general behaviour is necessary. This change must include both a different management of feeding behaviour and an increase in physical activity to leave up our sedentary lifestyle. If this is important for adult people presently overweight or obese, it is also important for adolescents. Many epidemiological studies has indeed shown that the obesity epidemic has extended to younger populations as indicated by an alarming increase in obesity prevalence in children with all bad associated health consequences when they will get older. Our genetic background has not changed over the last 20 or 30 years. The increase number of obese children shows us therefore that already in the very young age, epigenetic factors has become more and more important for the expression of genes that are considered as "bad" in our societies of abundance but that were probably "safe" or life-saving" in the pre-industrial times or farther in the time. It might therefore be assumed that lifestyle in the very early period of life is critical and must be acutely studied in order to propose a prevention policy.

In our animal experiments, we have studied the influence of unbalanced diets ingested by the mothers during the gestation and lactation periods. Both high carbohydrate and high fat diets were associated with different birth weights of the pups and several studies have focused on the importance of this parameter for a possible development of metabolic disorders in the adulthood. Supporting the "foetal programming" hypothesis, we have shown that important brain systems involved in the regulation of feeding were disturbed at weaning in the offspring (1, 2). These changes more particularly affected the systems that stimulate food intake. They are long-lasting as even in older adult animals, their functioning remained altered when stimulated in specific conditions.

The foetal programming was macronutrient dependent as growth curves of high carbohydrate and high fat offspring were not similar even during the suckling period. This might indicate that indeed during this period, the quality and quantity of milk produced by the dams and which depend on the composition of the ingested food is an important factor for development.

The early programming of the orexigenic systems has also lead to the establishment of different dietary preferences which could be of importance for adiposity development in

a context of food choice and large availability of food items.

Besides this "pure" energetic aspects based on diet composition, it appears from our most recent experiences (3) that the time scheduling of food ingestion including periods of restriction is also a critical parameter. Contrary to our expectations, we have found that food restriction during usual periods of eating in dams also leads to the installation of metabolic disorders (insulin resistance, adiposity) in the adult offspring. This factor appears as important as diet composition. Therefore it is obvious that both aspects must be considered in a policy of prevention.

In our mind, it is imperative to go on with research on all the factors that might affect the development in the foetal state and in the very early stages of life. The psychological factors such as stress or others must not be excluded as they are also important modulatory factors besides the nutritional factors. Knowledge is still too scarce on these aspects to propose 100% good counselling to the mothers. we think that only this preventive approach will be able to limit the expansion of the obesity epidemic.

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20ème Conférence Annuelle sur l'Obésité de l'AFERO – Nice (5-6/12/02)

Name: Wolf Endres

Title: Response to position papers by B. Koletzko et al. and S. Ozanne

Additional Thoughts & Literature: Most studies published before 1999 failed to demonstrate a significant effect of breastfeeding on the prevention of obesity (17). Following the reports by von Kries et al. some further studies have been published showing as well an inverse relationship between breastfeeding and the development of overweight and obesity in childhood and adolescence (2, 7, 13, 20). Thus, this relationship has been demonstrated in approx. 60.000 probands. However, there are also reports investigating more than 13.000 probands **not** supporting this relationship (9-10, 22). In an editorial Dietz (5) concluded that "the mechanism by which breastfeeding may protect against overweight or obesity remains uncertain. Infants who are breastfed may have more discretion over the amount of milk they consume than

those who are formula fed. The intake by formula-fed infants may in part be governed by the judgement of the person who is feeding the infant, who may prompt the infant to take more formula based on what he/she believes the infant should consume. ... The percentage of cases of obesity preventable by breastfeeding may be small." Butte (4) stated that "most studies examining the effects of breastfeeding on later obesity have found an insignificant effect. ... An effect of breastfeeding on later obesity, if any, is probably weaker than genetic and other environmental factors. Also, an observed association between breastfeeding and later obesity does not prove causality. ... In several of the later studies, adjustment for confounders obliterated the effect of breastfeeding. ... Although a highly provocative concept, the protective effect of breastfeeding on later obesity remains controversial." Martorell et al. (14) wrote in their review that the "literature is contradictory in part because many studies are based on small sample sizes and lack adequate control for confounding." There are many other factors such as obesity of the mother investigated in altogether 289.204 mother-child pairs (6, 21) as well as genetic influences (8) which have an important impact on the development of obesity.

Discussion: The underlying mechanism by which obesity in childhood and adolescence develops, seems to be multifactorial. Apart from the breastfeeding or not breastfeeding issue it may e.g. play a role which type of breastmilk substitute is fed, whether infants are forced to drink too high quantities, whether complementary foods are introduced earlier than in breastfed infants, whether the environment of an infant whose mother decided to breastfeed exclusively and/or rather long represents the attitude of a whole family which is more prone to result in a slim and healthy child than if he/she is bottle-fed, receives solid foods already during the first months of life, does have less contact to the mother who has to go back to work soon after delivery, etc. Mortensen et al. (16) "consider duration of breastfeeding as an indicator of the interest, time, and energy that the mother is able to invest in the child during the whole upbringing period. It may be that mothers who spend more time breastfeeding during the first year of life also spend more time later interacting with the child." The type of breastmilk substitute and of complimentary food fed might have a considerable influence on the development of obesity. In the context of Rolland-Cachera's work it is interesting to note that three studies have shown that the protein intake of infants and young children in Italy (3), Germany (12) and Denmark (15) is 2-3-fold higher than the daily amounts recommended by various nutritional societies. Most of the publications demonstrating programming effects are retrospective epidemiological studies. It's only recently that there are animal studies like that of Heywood et al. (11) demonstrating that the pancreatic glucokinase activity and insulin release can be programmed irreversibly by different protein quantities in utero and in the pre-weaning period with long-lasting effect throughout adult life. It has been shown that programming of obesity is possible by feeding milk formula high in carbohydrates to rats during their early weeks of life causing permanent changes in pancreatic islets and leading to normoglycemic hyperinsulinemia and development of obesity in adulthood (1). This metabolic programming carries through to the next generation: Offspring of these rats also developed hyperinsulinemia and obesity without any dietary modification (18-19).

Conclusion: The data published are contradictory, at least they do not allow to describe a lack of breastfeeding as an independent risk factor for the development of obesity in childhood and adolescence. However, they show that breastfeeding may have - among many other factors - a preventive effect on the development of overweight and obesity in later childhood and adolescence.

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Name: Ángel Gil

Title: Response to the position document on "Early Nutrition and Programming"

Obesity has reached epidemic proportions in developed countries. This alarming increase is present not only among adults but also among the youth. This pathology represents a serious threat to health because it increases the risk of developing many chronic diseases, such as diabetes and cardiovascular disease (1). In Spain, the incidence of obesity in children 6-14 years old has increased from 12.1% in 1980 to 16.2% in 1998-2000 (2). We have recently observed (FIS project N° PI 020826, Ministry of Health) that prepuberal children aged 6-13 years show higher arterial pressure, hypertriglyceridemia, lower levels of HDL-cholesterol and higher levels of plasma uric acid than aged- matched control children. Moreover, in these obese children, lower glucose tolerance and higher insulin resistance have been evidenced in response to a standard breakfast. These are risk factors influencing early onset of diabetes type II in childhood.

In the "enKid" Spanish study (1) a birth weight higher than 3,500 g was associated with a higher prevalence of obesity (OR 1.25) compared to those with a birth weight lower than 2,500g. In addition, artificial feeding during the first 4-6 months of life was related to a higher incidence of obesity in childhood and adolescence compared to children who were exclusively breastfed. Thus, breastfeeding might modulate later obesity risk..

Whether the reduced risk for overweight and obesity in breast fed infants are related to properties of human milk or to other factors i.e psychoaffective effects of breast feeding, remains unknown. In addition to significant differences in the levels of energy and protein of infant formulas compared to human milk, the quality of human milk fat, as well as the presence of more than 250 compounds (free amino acids, nucleotides and

nucleosides, sialyl oligosaccharides, gangliosides, hormones, growth factors, cytokines, etc.) which have significant biological activities, might influence not only the intermediary metabolism in early life but also have a role in the regulation of the expression of a number of genes involved in the control of dietary intake, energy expenditure, as well as adipocyte proliferation and differentiation.

Human milk contains high levels of oleic acid, essential fatty acids and relatively high levels of long-chain polyunsaturated fatty acids (LC-PUFA). Dietary fatty acids that are more prone to oxidation than to storage may be less likely to lead to obesity. In human, lauric acid is highly oxidised, whereas the polyunsaturated, and monounsaturated fatty acids are fairly well oxidised (3). LC-PUFA, particularly those of the n-3 series, play essential roles in the maintenance of energy balance and glucose metabolism. Dietary PUFA function as fuel partitioners in that they direct glucose toward glycogen storage, and direct fatty acids away from triglyceride synthesis and assimilation and toward fatty acid oxidation (4). Since LC-PUFA regulates the transcription of a number of genes implicated in lipid synthesis, fatty acid oxidation, and other relevant genes involved in the differentiation and metabolism of adipose tissue (peroxisome proliferator-activated receptor gamma -PPAR γ 2-, retinoic receptors, fatty acid binding protein aP2, and uncoupling proteins, UCP) the scientific community should evaluate the potential effects of human milk lipids and other dietary factors on the expression of relevant genes, which misregulation might be associated with overweight and obesity. The use of new technologies i.e. quantitative RT-PCR and evaluation of gene differential expression by microchips should be envisaged.

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Name: Claudio Maffei

Title: Early nutrition and programming research area -comments

Clinical evidence of the efficacy of increased skeletal muscle activity and diet modifications in promoting fat loss in obese individuals is available. At the same way, several studies clearly demonstrated that if lifestyle and nutritional changes are not promptly adapted at the new body weight and body composition, obesity relapse is almost inevitable. This suggests that once obesity is achieved a permanent return to a pre-obese state is very difficult. Similarly to diabetes, obesity is an efficiently self maintaining condition. The processes leading to excessive fat gain promote persistent functional and anatomical modifications in the body which guarantee survival at an upregulated homeostatic metabolic level till the system may sustain the effort.

The frustrating results of prevention and treatment of obesity, clearly suggest the need of identifying more sensitive targets for intervention. The impressive increase of the

prevalence of obesity suggests a prominent responsibility of the powerful pressure of the obesogenic environment in causing the phenomenon. Genetic susceptibility to high palatable and energy dense food and to reduced skeletal muscle activity is the necessary prerequisite. At the moment, dramatic environmental changes which may promote an anti-obesogenic environment are unrealistic. However, several studies support the hypothesis that early life (intra- and extra-uterine) and early nutrition may be a stimulating target for prevention.

Several factors, both genetic and environmental, affects foetal growth and body composition. Among these factors mother's health, behaviour (smoking, alcohol consumption, etc.) and nutrition were demonstrated to be particularly relevant. Sensitivity of the foetus to energy and nutrient deficiencies or excess in mother's nutrition changes in the different trimesters of pregnancy. The effects of malnutrition on the development of foetal central nervous system is largely unknown, especially the long term effects on the CNS areas (signaling pathways, etc.) and the neuroendocrine networks regulating appetite and satiety as well as body fat in extra-uterine life. Further studies on animals seem necessary to increase the knowledge on the physiology and physiopathology of the mechanisms regulating foetal metabolism and the programming of the long term regulation of fat mass in humans.

The macroscopic result of foetal malnutrition is given by the birth weight. A clear association between high birth weight and childhood obesity was consistently demonstrated as well as the high persistence (more than 50%) of childhood obesity into adulthood. The association between low birth weight and childhood obesity was not clearly proved whereas a rapid postnatal weight gain was associated with a higher risk of obesity. Low birth weight was consistently associated with type 2 diabetes, hypertension, and CVD in adulthood and the risk was increased in obese individuals. Therefore, infants born large (LGA) or small for gestational age (SGA) are by definition at metabolic risk in their future life. Operatively, the question is: a. to find the tools to promote an appropriate weight and body composition for gestational age in all the individuals; b. guarantee an appropriate growth velocity after birth on the basis of the future outcome of final weight, height, body composition and metabolic risk profile. Studies on animals are necessary to explore the efficacy and safety of an intervention on the mother during pregnancy to modify the growth and birth weight of the foetus and to evaluate the effects on body composition at birth of the different strategies of treatment. Some evidence of the role of breast feeding in reducing the risk of childhood and adult obesity is becoming available. The reasons of this finding are largely unknown although it is likely that the answer is in the macronutrient composition and hormones content of human milk. A few data are available on the role of complementary foods at weaning on growth velocity and obesity risk in children. Just a few data are available on nutrient metabolism in infants and young children and its relationship with obesity. Finally, investigation on the mechanisms of appetite regulation as well as on nutritional modulators of appetite in the foetus and infant is a priority of research.

Name: Kim Fleischer Michaelsen

Title: Response to position papers and comments on early nutrition and programming

Breast-feeding

Many studies have examined whether breast-feeding has an effect on the development of obesity later in childhood, adolescence or young adulthood. Two reviews have evaluated the evidence. In 1999 Parsson *et al.* published an extensive review of

childhood predictors of adult obesity¹, which also included a small review of infant feeding. The overall conclusion on breast-feeding was that there was no consistent pattern. Most of the studies measured obesity as an outcome in children below 7 years. Also, it was mentioned that many of the studies did not control for appropriate confounders. Later Butte published a more comprehensive review on the effect of breast-feeding on later obesity². The review included 18 studies. She found that in most studies there was no effect of breast-feeding on later obesity. Only four studies showed an effect. She concluded that if there was an effect, it was probably weaker than genetic and other environmental factors.

After these two reviews several large studies from Europe and USA, including the studies mentioned in the position paper by Koletzko *et al.*, found a protective effect of breastfeeding on childhood obesity³. However, some of these studies did not control for parental obesity, which might confound the association between breastfeeding and obesity, as mentioned by Koletzko *et al.*

Two small studies with individuals born in the mid 1940s have examined if there was a protective effect of breastfeeding on obesity in adulthood, but did not find an effect³. In a short report from a cohort study in New Zealand 1,000 individuals were followed from birth to 26 years⁴. Those breastfed for six months or more were less likely to be overweight at 26 years, but the association was weaker and non-significant when controlling for confounders including parental overweight.

We have studied the effect of breastfeeding on adult obesity in the Copenhagen Perinatal Cohort, which consists of 9,125 individuals born 1959 to 1961⁵. The cohort is described in more detail in a recent paper in which we found that the duration of breastfeeding was positively associated in a dose-dependent manner with IQ in young adulthood⁶. In a sub-sample of 2,601 males the following information was available: Duration of breast-feeding assessed by a questionnaire at 12 months and weight and length at draft board examination at 19 years. Although the infants who were breastfed were leaner at 12 months, breast-feeding did not protect against overweight (BMI>25) or obesity (BMI>30) as young adults.

In conclusion, it is still not clear whether breastfeeding protects against obesity. The available studies make it plausible that there is an effect, at least in some settings during certain ages. Recent large studies showing an effect in young children suggest that the effect is stronger when the prevalence of obesity is high, as in the current epidemic of obesity. However, breastfeeding is not likely to explain a major part of the obesity epidemic. In several European countries, like the Scandinavian countries, there was a dramatic decrease in breastfeeding rates until 1970 after which there was a steep increase. This does not match the steady marked increase in the prevalence of obesity during the last 3-4 decades.

If there is an effect of breastfeeding, then there are several potential mechanisms. Understanding the mechanisms is important, even if the effect is small or transient, as this may help us understand the causes of the current epidemic of obesity. As pointed out by Koletzko *et al.* these could be differences in protein intake, or bioactive factors in human milk that could act through a modulation of the hormonal profile in the offspring. Other potential explanations could be a better satiety regulation in breastfed infants. Breast milk composition and taste reflect the diet of the mother and changes considerably over time. This provides the infant with much more composite signals than the bottle-fed infant, and gives the breastfed infant a more active role. It is speculated that this could give a better satiety control later in life. Furthermore, it is possible that

mothers who breast-feed are on average more caring and supportive during later childhood, which may have a protective effect against obesity. In a Danish study parental neglect in school-aged children was a very strong predictor for development of obesity in young adulthood⁷.

Early growth

Birth weight has been shown to be positively related to subsequent adult obesity in many studies. However, in an interesting study based on the 1958 British birth cohort by Parson *et al.* maternal weight or BMI largely explained this association⁸. It was also shown that rapid linear growth before the age of 7 years increased the risk of obesity in adulthood. This is in line with other studies suggesting that catch-up growth during early postnatal life has adverse effects on the risk of developing non-communicable diseases later. Thus, intrauterine and early postnatal growth (linear growth, weight gain and changes in body composition) seems to be important for the development of later obesity. However, our understanding of what regulates and programmes growth during early life and childhood is limited. Several studies have shown that the obesity epidemic is also affecting young children suggesting that factors early in life are involved. In a Danish study, presented at the 9th International Congress on Obesity, Brazil, 2002 it was shown that the prevalence of obesity in 7 year old school children increased more than 6 fold (from about 1.5% to 10%) from 1936 to 1975⁹. The analysis was based on measurements of more than 250,000 children. During the same period birth weight was stable, showing that the epidemic developed after birth and before 7 years. In a British study there was a highly significant increase in weight and body mass index from 1989 to 1998 in children under 4 years of age¹⁰. Overweight increased from 5.4% to 9.2%. Thus, it seems like the obesity epidemic starts in early childhood. Although reduced physical activity and a high-energy intake could also play a role during this age, it is not likely to be the main causes. Several studies did not find significant associations between fat intake and weight gain in young children. It seems more likely that other early exposures are important causes of the obesity epidemic.

The Danish National Birth Cohort (Better health for mother and child) includes 100,000 women who were recruited early in pregnancy¹¹. The aim is to register exposures during pregnancy and infancy to examine long lasting impact on health and disease. Exposure information is mainly collected by computer assisted telephone interviews twice during pregnancy and when the children are 6 and 18 months old. The telephone interview includes growth data based on measurements made by the family physician entered in a family kept record. The mothers are also asked to fill in a self-administered food frequency questionnaire in mid-pregnancy. Furthermore, blood taken from the mother twice during pregnancy and blood from the umbilical cord are stored in a bio-bank. Recruitment started in 1996 and was completed in 2002. This cohort gives unique possibilities for examining the effect of exposures during pregnancy and infancy to early the early growth pattern. Furthermore, the effect of early growth and early exposures on later development of obesity can be examined in follow-up studies.

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Name: Luis A. Moreno

Title: Response - Early nutrition and programming"

Introduction

In my opinion, the importance of the obesity epidemic would be justified not only in terms of elevated prevalence in the US, but also in Europe, even in children (Moreno et al, 2000; Chinn & Rona, 2001; Bundred et al, 2001; Rudolf et al, 2001; Kautiainen et al; 2002). With higher prevalences in the south than in the north of Europe.

In European obese children and adolescents it seems that type 2 diabetes mellitus is not so high than in some US populations (personal communications of different paediatricians from obesity clinics). However, it seems that European obese children and adolescents show a clustering of cardiovascular risk factors that could be considered as the metabolic syndrome (Csábi et al, 2000; Moreno et al, 2002).

Metabolic imprinting of later obesity

Dr Ozanne and Prof Koletzko et al consider, as a departure, the link between low

birthweight and later obesity; however, some studies have shown that heavy newborns tend to become fat adolescents and adults (Seidman et al, 1994; Curhan et al, 1996). Frisancho (2000) has also observed that heavy newborns (assumed to be fat newborns) do not necessarily become fat adolescents unless the mother or father is also overweight.

Some authors have observed a significant relationship between low birth weight and increased central fat deposition in adolescents, assessed by skinfolds ratios (Barker et al, 1997, Malina et al, 1996), and central obesity in adulthood, assessed by the waist-to-hip circumference ratio (Law et al, 1992). Okosun et al (2000) have also observed a negative association between birth weight and subscapular skinfold and central adiposity in White, Black and Hispanic American children aged 5-11.

Low stature in the neonatal period is also considered a marker of intrauterine nutrition. Sichieri et al (2000) has observed increased risk of obesity and abdominal fatness among women with newborn low length.

Dr Ozanne points on the need to implement research programs aiming to investigate catch-up growth; however, in my opinion, I think that infants that catch-up are those that need to do it... It would be more adequate to investigate and to try to prevent the different causes of defective nutrition in the foetal and early life.

Metabolic imprinting of type 2 diabetes and cardiovascular disease

The importance of obesity as a public health problem is justified by its consequences. That is the reason why, in my opinion, it would be interesting to consider the metabolic imprinting not only in terms of obesity in later life, but also in terms of its consequences.

Studies in both children and adults of various races have shown a consistent association between features suggesting impaired foetal growth and measures of insulin resistance (Phipps et al, 1993; Law et al, 1995). A number of hypothesis have been proposed to explain these links with low birth weight. The authors of the original low birthweight-adulthood disease associations proposed that maternal-fetal undernutrition could result in long-term change, or programming, in metabolic or hormonal activity in the offspring, for example involving insulin and the insulin-like growth factors (Hales & Barker, 1992).

Hattersley & Tooke (1999) have proposed that the association between low birthweight and adult insulin resistance is principally genetically mediated. Genetically determined insulin resistance could result in low insulin-mediated foetal growth in utero as well as insulin resistance in childhood and adulthood. Central to this foetal insulin hypothesis is the concept that insulin-mediated foetal growth will be affected by foetal genetic factors that regulate either foetal insulin secretion or the sensitivity of foetal tissues to the effects of insulin.

Both hypotheses provide plausible explanations for the associations seen between foetal growth and adult disease, and both are likely to have a role.

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Name: Claude Remacle

Title: Response to the Position paper on "Early Nutrition and Programming"

There is no doubt that research on early determinants of obesity is a crucial issue in view of the dramatic increase of the disease over the world. Indeed, an important cause may be the long-term programming by nutrients available to the foetus and newborn. Clearly, a large body of arguments exists to sustain the foetal origin of syndrome X, as related to the thrifty phenotype hypothesis. It is certainly true for the occurrence of glucose intolerance, type 2 diabetes, and cardiovascular disease. Several epidemiological studies as well as research using animal models substantiated the link between low birthweight and later anomalies in structure and function of insulin-producing cells as well as peripheral tissues sensitive to pancreatic hormones. Conversely, the progeny of diabetic mother or dam features abnormalities in these tissues which may lead to transmission of metabolic alteration from generation to generation.

When focusing on early programming of obesity as such, the argumentation is lighter and appears more difficult to gather. For example, in the Ravelli's study on the cohort issued from Dutch Famine, those who were exposed early in their intrauterine life to the famine did not have lower birth weights, but they were prone to becoming obese later on. In contrast, those exposed towards the end of gestation had lower birth weight and showed a higher rate of impaired glucose tolerance, while having a lower risk of obesity. In the same study there were also different consequences on the appearance of obesity according to gender, only women being affected by general obesity at the age of 50. Another source of complication for determining the relation between foetal growth and obesity in later life is the possible "U-shaped" form of the relation. In addition to the "light baby – obese adult" hypothesis, several studies showed that people who were heavy at birth or in young age tended to be more obese as adults. Finally, studies presently conducted on infants *i.a.* in UK and in India provide evidence that children that experienced early catch-up growth have a higher propensity to develop intra-abdominal adiposity. This is particularly relevant to syndrome X, since this depot is recognized as a main provider of molecules involved in hypertension and pathogenesis of cardiovascular diseases.

Then, there is an urgent need to clarify the relation between foetal and neonatal growth and the appearance of obesity, both with epidemiological approach and the use of animal models. For the latter purpose, the selection of altricial or precocial species may be of importance, since the extend to which various components of syndrome X emerge postnatally after perinatal perturbation may relate to the maturity of the species at birth. Many body systems undergo sensitive or critical periods after birth in altricial species and before birth in precocial ones. The notion of critical window for programming is implicitly included in the search for mechanisms at the tissue or cellular level. It is

conceivable that most critical repercussion will result from alteration of the (nutritive) environment during times of most active proliferation, commitment, differentiation, and setting up of intercellular communication.

Programming of obesity may occur at the level of one or more of different organs. The adipose depot itself, since the adipocyte maturation is the last step of a process of multiplication, commitment and differentiation of fat cell precursors, of which the population may clearly be influenced by both general and specific nutrient availability. Another level of programming is the hormonal context, since adipocyte differentiation and function are regulated by a large series of growth factors, hormones and cytokines. A third level is the central mechanisms regulating food intake and energy expenditure. All these will merit considerable attention.

Name: Dr Martin Wabitsch

Title: Response to the "Early nutrition and Programming" research area

General:

The three research areas which will be discussed during this Workshop are well selected. There are research groups in Europe of high quality which will be able to work in these areas successfully. However, other important areas are not addressed and I propose that an area 'Influencing environments to reduce obesity prevalence' could be another important research area in Europe. There is obviously an imbalance between basic obesity research and research related to action: Millions of Euros are provided for research related to genes, metabolism, drugs and market lobby, and high tech and only small steps are taken to improve knowledge on successful actions against the increase in obesity prevalence in European countries by research projects related to environment, public policy, community, and prevention. This should be considered when aiming at increasing the impact of European Obesity Research.

Specific:

A specific research objective which should be addressed in relation to 'Early Nutrition and Programming' and 'Genomics and Biotech' is adipose tissue development and function. This would be important for the understanding of the development of obesity and its comorbidities. Briefly, I would like to outline some aspects:

There are well defined periods during fetal life, infancy, childhood and adolescents with different patterns of growth of lean and fat tissue. Specific sensitive periods for adipose tissue growth have been identified. Body fat content is around 13 % and rises to 28 % by the end of the first year in a normal-weight infant. This period of adipose tissue growth is followed by a period during which the subcutaneous adipose tissue mass decreases along with the percentage of body fat due to relative higher increases in lean body mass during growth. Interestingly, during this period the longitudinal growth is not accelerated. Later these cyclical changes are repeated, with percentage body fat increasing between the eighth and tenth years of life, in early puberty.

On the basis of results derived from investigations of adipose tissue cellularity and of thymidin kinase activity in the stromal-vascular fraction of adipose tissue two sensitive periods for adipose tissue development during childhood could be identified: one during the first year of life and another one just before puberty. It seems that during the first year of life the increase in body fat content is mainly due to an increase in adipocyte volume. The further growth of adipose tissue is suggested to be mainly due to an

increase in fat cell number without significant changes of fat cell volume. Changes in adipocyte number (increases or decreases) are the result of either a multiplication of preadipocytes and their subsequent differentiation into adipocytes resulting in an increase in fat mass or of dedifferentiation or apoptosis of existing adipocytes leading to a loss of adipose tissue mass.

Environmental factors interfering with the regulation of adipogenesis in these sensitive periods as well as the susceptibility of adipogenesis for allowing changes induced by these factors may determine the growth rate of the adipose organ. This fact is also demonstrated by the observation that differences between the subgroups with different infant nutrition patterns (breast feeding vs bottle feeding) only evolve after the fourth year of life and this is just after a sensitive period of adipogenesis. Moreover, it has been shown that the time of 'adiposity rebound' is critical for the development of obesity or in other words might be a very early sign of developing obesity.

There are different ways to identify and investigate hormonal and nutritional factors involved in the regulation of adipose tissue growth in these sensitive periods of adipogenesis. Studies on adipogenic activity in human serum are carried out using cultures of preadipocytes as an in vitro model. It has already been shown that glucocorticoids and IGF-I are major determinants of the activity of human serum to promote adipocyte differentiation. The role of retinoids and fatty acids in the control of adipose differentiation has to be clarified. TGF α , TNF α , EGF and PDGF are suggested to contribute to the anti-adipogenic activity of human serum. There are certainly more yet unknown factors in human serum which are able to inhibit and therefore control adipose differentiation in man. Another approach is the study of the regulation of proliferation and differentiation of preadipocytes directly after stimulation with specific factors under chemically defined conditions. This approach has revealed that the control of adipogenesis in man is different to that in rat or mouse. Therefore, data obtained with mouse-derived preadipocyte cell lines can not directly be taken over for the physiology in man. Recently, human preadipocyte cell lines with high capacity for adipose differentiation have been established in European research labs.

The phenotypical and functional changes which occur during the differentiation of a preadipocyte into an adipocyte are the results of molecular changes related to changes in the gene expression pattern. The differentiation process is characterized by an increase in the expression of adipocyte-specific genes (e.g. LPL, GPDH, Glut 4, Leptin etc) and the decrease in the expression of genes being predominant in preadipocytes (e.g. preadipocyte factor-1). Changes in gene expression are regulated by transcription factors. In the last few years such "key regulators" of adipocyte gene transcription have been described. It is known today that a combination of factors from the C/EBP-family and the PPAR-family will positively activate adipocyte gene transcription. PPAR isoforms have natural ligands such as fatty acids, prostaglandines or leukotrienes or related metabolites. Further knowledge of the role of such natural ligands in activating PPARs and thus the transcription of adipogenic genes, will help to better understand the link existing between the fatty acid composition of food and the formation of new fat cells.

Furthermore, adipocytes are secretory cells producing many factors involved in the pathophysiology of obesity-related comorbidities. Investigating and influencing the production of these factors in adipocytes could be another target for drug development.

Research work producing the above mentioned results has mainly been carried out in European Research Groups. These groups could therefore form a competitive network

in the future and could significantly contribute to the development of a European Research Area on Obesity by rising research projects related to 'Human adipose tissue growth and function'.

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