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About CSCI

MISSION

To promote clinical and basic research in the field of human health throughout Canada, to lobby for adequate research funding at the federal, regional and local levels, and to support Canadian researchers in their endeavours and at all stages of their careers.

The Society still fulfills its original mandate today. It has evolved, however, to include the active promotion of clinical science and lobbying for support of basic and applied biomedical research from the federal and provincial governments. CSCI members represent researchers across Canada who are studying issues of disease and health care across the spectrum, from basic research to issues of health care delivery.

ORIGINS

The Canadian Society for Clinical Investigation (CSCI) was founded in 1951 and its original purpose was to provide a forum for the exchange of scientific information. It was envisaged as a "travel club for those interested in clinical investigation in Canada". As detailed by J.S.L. Browne, one of the four founding members of the CSCI, the idea was for it to be a very informal organization and not a society.



Its first meeting was attended by 44 people and was an outstanding success. Over the next several years, discussion continued as to the proposed nature, structure and organization of a society for Canadian clinical investigators. These discussions culminated in the formation of the CSCI in 1959. Its first meeting was held in Vancouver that year and the meetings have continued to grow in size and are now held conjointly with the annual meeting of the Royal College of Physicians and Surgeons of Canada.

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The CSCI is composed of individuals interested and active in clinical investigation from across the country. Membership is open to those who are interested and active in clinical research and who are sponsored by a member of the Society.

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The Canadian Society for Clinical Investigation is pleased to request submissions for the following awards:

Deadline March 31, 2006: CSCI/RCPSC/CFBS G. Malcolm Brown Lecture

Deadline June 26, 2006: CSCI/CAPM Core Medical Residents Research Award

Deadline July 7, 2006: CSCI/CIHR Resident Research Prize

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The beginning of one's real ethical development.

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" To care for anyone else enough to make their problems one's own, is ever the beginning of one's real ethical development".¹

- Felix Adler, founder of the New York Society for Ethical Culture, 1876.

Ethical conduct is an integral component of medical research and yet its fundamental concepts are rarely discussed until a dilemma arises. In an effort to foster the understanding of ethical concepts among trainees in the Clinician Investigator Program (CIP), the Faculty of Medicine and the Joint Centre for Bioethics, University of Toronto recently held the annual CIP Research Ethics Day. The introductory address was given by Dr. Solomon Benatar, Professor of Medicine and Director of the Bioethics Centre, The University of Cape Town, South Africa, who spoke about social relations, ethics and international research. Dr. Benatar highlighted the ethical tensions inherent in conducting research to advance knowledge while avoiding the exploitation of potentially vulnerable participants in developing countries. To prevent the exploitation of research participants in developing countries, Dr. Benatar proposed:

- 1) clinical trials should be relevant to the health needs of the host country;
- 2) priority should be given to trials that will benefit the host country;
- 3) members of the host country should be involved in the design and conduct of trials;
- 4) researchers must respect the dignity of participants by obtaining meaningful informed consent;

5) care should be provided for diseases other than those being investigated in the research study; 6) study findings, where possible, should be incorporated into local health care systems;

7) alliances must be made to promote sustainability of health care after termination of the research project. Greater detail can be found in Dr. Benatar's article "Towards progress in resolving dilemmas in international research ethics".²

Research Ethics Day participants were then divided into smaller groups to discuss specific ethical topics in greater detail. The first session, "Informed Consent: can obtaining it ever be unethical?", began with a review of basic ethical principles (autonomy, beneficence, non-maleficence and justice) and the necessary elements of informed consent, as outlined in the "Tri-Council Policy Statement: ethical conduct for research involving humans".³ Participants were then asked to appraise critically two research proposals as if they were members of a Research Ethics Board (REB). The first outlined a randomized controlled trial in which patients with severe head injuries were assigned to receive either mannitol or an undisclosed drug, "Drug X". The investigators claimed that informed consent should be waived given the incapacitated nature of the subjects and their need for emergency treatment. The key issue raised by the mock REB was the requirement for a state of clinical equipoise between mannitol and "Drug X" if informed consent was to be waived.

The second research proposal outlined a prospective study looking at the quality of life of patients

dying from metastatic lung cancer. The investigators claimed that full disclosure of the intent of the study was not required because reminding patients of their fate could cause them harm. The mock REB determined that the critical issue to consider was the likelihood of subjects suffering severe distress as a result of this disclosure. At the end of this session, participants became aware that REB members rely heavily upon the principal investigator's knowledge and critical evaluation of the literature when considering whether or not informed consent can be partially or completely waived.

The second session, "Research Ethics Board: who wants a million dollar REB application?" used a game show format to discuss the function of an REB, and the key ethical issues that REBs address. Questions were posed to contestants, and four possible answers were provided. Participants who correctly answered their question then moved on to the next, more difficult question. If unsure of the answer, contestants had an opportunity to use one of the following "life-lines"-ask a friend or poll the audience. After each correct answer, an explanation was provided and an opportunity was given for audience members to ask questions. Participants continued to play until they answered a question incorrectly, at which point they returned to their seat and a new contestant was selected to play the game.

The game show format accomplished several objectives:

- 1) It involved all members of the audience, including some who otherwise may not have participated,
- 2) It stimulated camaraderie and a friendly spirit of competition,
- 3) It transformed material that might be considered, by some, to be dry and voluminous, into more enjoyable and easily-manageable chunks.

The third session, "It's Publish or Perish: considering the ethics of research paper authorship", used case scenarios to explore considerations regarding inclusion of authors and order of authorship. Following this, participants reviewed the "Uniform requirements for manuscripts submitted to biomedical journals" by the International Committee of Medical Journal Editors.⁴ Conclusions drawn from this session were:

- 1) authors must contribute to the conception, design or analysis of data, drafting the article or revising it, and final approval;
- 2) all individuals who participated sufficiently should be recognized;
- 3) authors should outline their individual contributions to the manuscript upon submission;

4) each author should have participated sufficiently to take public responsibility for its content;

5) order of authors should be the joint decision of the coauthors. The session concluded with consideration of the ethical values that influenced authorship decisions, such as justice, veracity, avoidance of exploitation, and responsibility.

The final session, "Of Genes and Drugs: ethical considerations in pharmacogenetics", explored the role of heredity in person-to-person differences in drug responses and adverse effects. Research in pharmacogenetics has introduced the concept of personalized medicine - getting the right medication to the right individual at the right dose. Historically, pharmacogenetic analysis was conducted predominantly under the auspices of academic institutions. However, this research has recently shifted into the domain of the pharmaceutical industry because adverse drug reactions are now a leading cause of death worldwide.⁵ This shift in regulation from academia to industry has led to an evolving set of ethical considerations. Participants were asked to pretend they were members of an advisory committee hosted by a pharmaceutical company that recently developed a new medication for treating migraine headaches. More specifically, participants pretended they were major stakeholders (researchers, REB, study participants, pharmaceutical industry, regulatory bodies and global health organizations) who each had their own vested interest in the development and testing of the drug in question. Based on review articles by Mordini et al.⁶ and Lipton⁷, the group determined the most important issues to be:

- 1) a governing body is required to regulate the field of pharmacogenetics;
- 2) this body must determine the extent to which pharmaceutical companies should have access to genetic information;
- 3) legal and ethical standards must be devised to regulate sharing of genetic information;
- 4) individuals who donate genetic material to develop a test should receive feedback regarding their results;
- 5) the potential for inequalities in the provision of healthcare must be addressed;
- 6) the financial burden incurred by genetic testing in the development of pharmaceutical agents must be evaluated.

All sessions were well-attended and received excellent evaluations at the end of the day, demonstrating that ethical concepts can be discussed in a meaningful manner even before a real life dilemma arises.

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Annual Incidence of type 1 diabetes in Québec between 1989-2000 in children.

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Polychronakos C, MD,

Division of Endocrinology and Metabolism, Montreal Children's Hospital, McGill University, Montreal, Canada. Presented in part in a poster form at annual Canadian Diabetes meeting in October 2002, Vancouver, Canada

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Abstract

Purpose: To document the number of new pediatric cases of type 1 diabetes diagnosed each year, from 1989 to 2000, in the province of Québec. To analyze secular trends and age of presentation during the same period.

Methods: Data, gathered through a government allocation program, provided the number of reported new cases. The data bank also made available the age at diagnosis, sex and geographic distribution of cases.

Results: A steady number of new cases, approximately 240 p.a., was diagnosed over the 12-years. The annual incidence in the pediatric population of Québec was 15 per 100,000. There was no trend towards earlier age at diagnosis.

Conclusions: We found no evidence of increase in the number of children diagnosed with type 1 diabetes in Québec between 1989-2000. Also, over the same period, the data did not support a younger age at diagnosis.

The incidence of diabetes is apparently increasing everywhere. This comes at a time when type 2 diabetes is being screened for more systematically in children, the obesity epidemic having put this once-thought rarer form of diabetes in the forefront.¹ While this might have created a climate of confusion,

evidence that type 1 diabetes is also on the rise is available. The best documentation comes from the Scandinavian countries²⁻³ where increases (on average 30%) have been documented over the last 10-15 years but this may be reflecting a general trend.⁴⁻⁵ The diagnosis is also believed to arise in younger children.^{2,6} We sought to determine the annual incidence of type 1 diabetes in the province of Québec (2nd largest in Canada) over a 12 year span (1989-2000) to establish recent incidence data. The last official data of 1983⁷ established for the Montreal area (largest city in the province) an average annual incidence of 9.3/100,000. We were also able to look at regional distribution of cases and age at diagnosis. This work will establish the current annual incidence data for the province of Québec and allow us to comment on its secular trends.

Research design and Methods

The province of Québec is home to about 7 million individuals. Every new diabetic's family who resides in the province is allowed a family allowance supplement under the Régie des Rentes du Québec program. This supplement is available to every child under 18 years old at diagnosis regardless of family income and represents a considerable incentive for reporting cases. Thus, the data collected represents incidence rates in the 0-18 year old age group that is different from most reports which refer to the 0-14 year old group.

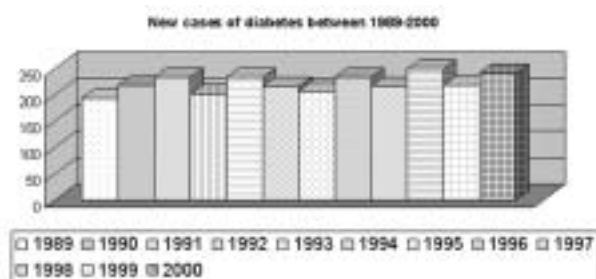


FIG.1 Annual incidence of type 1 diabetes in the province of Québec, 0-18 yr, 1989-2000.

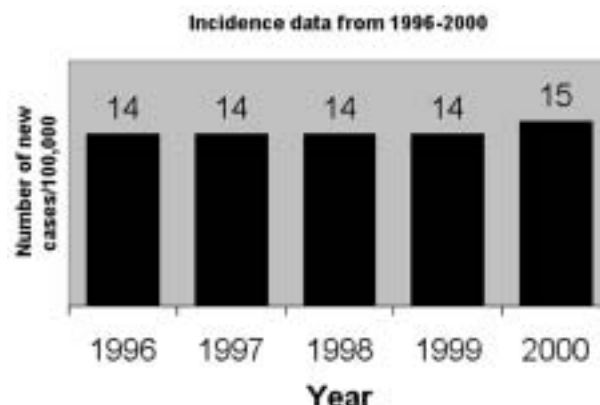


FIG.2 Trends in annual incidence from 1996-2000 in the pediatric population in Québec.

The treating physician, upon diagnosis, is invited to fill in the forms and the data is gathered at the Régie and has, since 1989, been collected in a computer-based data bank. Contacts were established with the data bank managers and information was released to preserve anonymity. The data reveal age at diagnosis, geographic distribution of cases and sex of the affected child. Information from 1989-2000 allowed us to document trends over a 12 year span. From 1996 to 2000, annual incidence data could be established by age groups and sex, the data being reported by the Régie per 100,000 children. This information was not available before 1996 due to different data collection procedures. No major changes in total population or regional distribution occurred between 1989-1996.

Chi Square analysis was used to compare raw data for 1989-1995 and incidence rates for 1996-2000, to document differences in trends over time and in all age groups. Incidence data were also compared between regions to establish homogeneity of the distribution of cases. Cross-validation was attempted through a survey amongst the four major university centres whose referral areas cover two thirds of the province's children affected with type 1 diabetes.

This project was approved by the "Commission d'accès à l'information", the government agency superseding control of data circulation potentially leading to breach of privacy.

Results

From 1989 to 2000, the number of new cases across the province was remarkably steady, around 240 cases a year (Figure-1). Fifty three percent were male, (P=0.03), and this was steady over time. However the

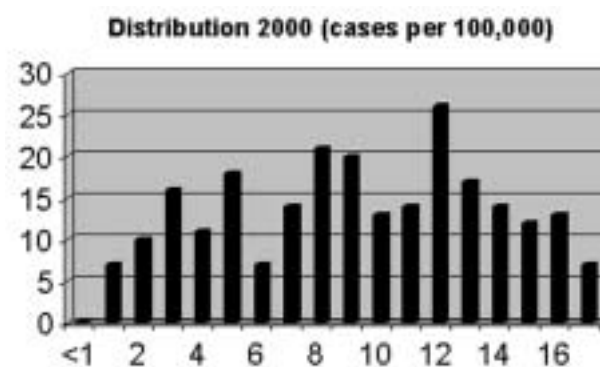


FIG.3 Annual incidence, new cases per 100,000 children, of type 1 diabetes in Québec in different age groups for 2000.

latest annual incidence of 15/100,000 (Fig-2) is higher than the data of 1983.⁷

Peaks of incidence are seen in the 13 year old category (25 per 100,000) and the minimum incidence is seen in <1 year olds (1 per 100,000) (Fig.3). Looking more specifically at younger children, preschool children (<5 years) represent 25% of cases with no evidence of increase, either as a group or by age (Fig 4). While an increase in incidence was documented in the 2-3 year olds (from 13 to 15 per 100,000), this was not statistically significant.

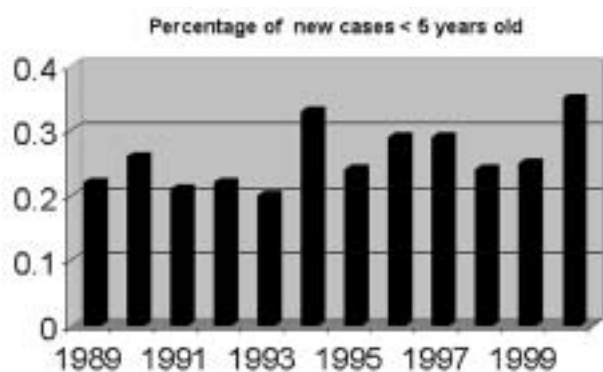


FIG. 4 Percentage of new cases, < 5 yr, diagnosed in Québec, 1989-2000.

Geographical differences among regions were found by comparing regions of origin of the cases reported. Some remote regions reported more cases per capita than the more populated regions. Regional incidences varied from a nadir of 0 (no cases reported) to 24/100,000. The administrative area of Montreal has an incidence of 14/100,000.

Cross-validation of the data corroborated these results. Based on local patient census, none of the four university centres showed any increase in the number of new type 1 diabetics during the same period (1989-2000).

Discussion

The major finding of this study is the establishment, through physician reported data, of a stable annual incidence for type 1 diabetic Québec children over a 5-year span (1996-2000). The most recent (i.e. 2000) is 15/100,000. This is similar to the incidences in the United States and the U.K.⁶ and is higher than the previously reported incidence of 9.3/100,000 for the Montreal area.⁷ The incidence in the younger age groups (< 5 years old) is stable over the same span. This corroborates the findings of the major university-based centres that cater to two thirds of these patients as well as to the absolute number of new cases which has been steady for 12 years (1989-2000). A predominance of boys is frequently reported in countries where the incidence is higher than average.³ There is no explanation for this phenomenon.

The 1983 survey, on which the official Montréal data are based, was gathered through a review of a representative sample of Montréal area hospitals' admission

records.⁷ The incidence rates were gathered on children up to 14 years old, which is the standard way of expressing incidence data. The incidence calculated in 2000 is not different whether we look at the 0-14 or the 0-18 year old groups. Siemmiatycki et al presented a survey only of the greater Montréal area. Extrapolating that data province-wide suggests that the incidence does not vary across the province which, from our data, is a false assumption and is frequently seen in other settings. Reported incidences are known to be different across the country.⁶ A recent Norwegian study reported regional differences but with no increase in overall incidences.⁸ Another reason for different incidence rates between the two studies was the multiplicity of centres outside the catchment area where some of these children may have been admitted. Given the methodologic differences between the two studies, we conclude that there is no evidence of a steep increase to explain the sudden change in incidence between 1983 and 1989. Moreover, we have documented a remarkably steady number of new cases from 1989 to 2000. This includes the younger age group, whether analyzed as a whole (i.e. <5 year old) or as individual age matched sub-groups.

From a review of incidence data from many countries, it is clear that this phenomenon is not seen in all areas (Montreal).⁵ Several reports have come from Europe. There are fewer North American reports but a recent review from Philadelphia revealed an increase probably linked to a rubella epidemic.⁹ Such regional differences support the hypothesis that environmental triggers are responsible for some of these trends. Data from the neighboring province of Ontario point to an increase of prevalence, not of incidence of diabetes occurring over the same period.¹⁰ There is still confusion about whether some areas are witnessing an increase of incidence or prevalence. For the period analyzed, our data do not support a provincial increase in incidence. Furthermore, there are no explanations for the increase witnessed in other countries. This would favour a widespread, shared environmental trigger which remains unknown. Two recent papers from Belgium and Sweden suggest this could only reflect the emergence of the disease earlier in life as the overall incidence of type 1 diabetes in the <40 year old group remained the same.¹¹⁻¹² One may speculate whether this could be the case for other countries. This data was not available for our group.

While these data are reliable, there are some limitations. First, reporting by physicians may not have been complete. However, a recent meeting of pediatric dia-

betes caregivers in Québec failed to reveal incomplete systematic completion of these forms because of the powerful financial incentives. Reporting practices should not have changed over time. Second, the actual incidence rates are only available for 1996-2000, but the raw data demonstrate a steady number of new cases dating back to 1989. If one speculates that the number of children remained steady or declined slightly between 1989-1995, then the incidence can be estimated to have remained steady. That information was unfortunately not available as census data are only gathered every five years in the province. The possible confounding type 2 factor was unlikely before 1996 when this diagnosis in our population was almost unheard of. Finally, the diagnosis of diabetes for the purposes of the Régie's reimbursement is reviewed by a committee including a pharmacist or a physician and refers to insulin treatment before initiating payments. Confounding possibilities are thus limited.

In conclusion, further work is needed to understand better the increases in incidence seen in different settings. This data bank has helped to establish a more likely incidence rate for type 1 diabetes in Québec in recent years. The incidence is 15/100,000 and has remained steady from 1996-2000. The age at presentation has not changed. Because we can no longer rely on this data bank, establishment of a province-wide diabetes registry could help us track changes over time. Work is under way through a province-wide pediatric diabetes network to improve gathering of epidemiological data as well as to encourage collaborative research.

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ORIGINAL RESEARCH

Cholesteryl ester transfer protein gene polymorphisms and severity of coronary stenosis

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Abstract

Purpose: Cholesteryl ester transfer protein (CETP) gene is involved in the reverse cholesterol transport in humans. Thus, it is a candidate for studying the susceptibility to coronary heart disease (CHD). The goal of the current investigation was to determine any association between CETP polymorphisms (I405V and TaqIB), and severity of coronary stenosis, since the extent of coronary artery narrowing has been considered as a primary determinant of survival in CHD patients.

Methods: The severity of coronary stenosis was estimated by Gensini (GS) and Duke Jeopardy (JS) scores in 130 men with documented CHD (mean age 61±10 yr). Genotyping was performed by polymerase chain reaction and restriction fragment length polymorphism analysis.

Results: The allele frequencies for both TaqIB and I405V were found in Hardy-Weinberg equilibrium. Homozygotes for I had lower GS compared with heterozygotes (IV) and homozygotes for V [72(95%CI 51-92) vs. 122(95%CI 88-157) and 136 (95%CI 74-198), P=0.009, P=0.010, respectively]. In addition, GS differed between carriers of I and carriers of V allele [86(95%CI 72-101) vs. 128(95%CI 102-154), P=0.003]. A correlation was found between the GS and I405V polymorphism (r=0.250, P=0.005), but not with JS.

Conclusion: Homozygosity for I405 favours less severe coronary stenosis. Our findings indicate that the I405V polymorphism may have potential importance in screening individuals at high risk for developing CHD, establishing efficient prevention measures, and searching for other risk factors for CHD. However, further prospective investigations in larger populations are required to confirm these findings.

Since the discovery of cholesteryl ester transfer protein (CETP) and its identification as a modulator of high density lipoprotein cholesterol (HDL-C) levels, there has been much speculation about its role in human disease.^{1,2} High levels of HDL-C are well known to be protective for cardiovascular disease but the uncertainty surrounding the detailed mechanism(s) for HDL's advantageous effects has led to controversy. A question rises whether the high HDL-C levels, induced either by genetic deficiency of CETP, or by therapeutic inhibition of CETP would be beneficial. Animal model studies have been of limited value in addressing the question, because many species do not express a functional CETP protein, do not normally carry the bulk of their cholesterol on low density lipoprotein (LDL), and do not develop atherosclerotic lesions over the same time frame as humans. Thus, most animal models are not

reliable to provide strong evidence for CETP's role in disease. The CETP gene, located on chromosome 16q21, consists of 16 exons and spans a region of ≈ 25 kb.^{3,4} Several rare mutations that result in the absence of detectable CETP mass and/or activity have been reported at the CETP gene locus. In humans, CETP deficiency is characterised by the presence of increased concentrations of large, cholesteryl ester-enriched HDL particles in the plasma and, often, reduced concentrations of LDL cholesterol (LDL-C).⁵ The former is the result of delayed catabolism of cholesteryl ester-enriched HDL particles,⁶ whereas the latter is due to accelerated catabolism of triglyceride-enriched LDL particles.⁷ In addition to rare mutations, several common polymorphisms have been identified in the CETP gene. Several polymorphisms identified in the coding sequence of the CETP gene include I405V⁴ and R451Q.⁸ The I405V variant is associated with reduced CETP mass and increased HDL-C levels.^{9,10} Another widely studied CETP variant is TaqIB, a silent base change affecting the 277th nucleotide in the first intron of the CETP gene.¹¹ In normolipidemic subjects, absence of the TaqIB restriction site (B2 allele) is associated with decreased CETP activity and, in turn, increased HDL-C levels,¹²⁻¹⁴ resembling a mild form of CETP deficiency. The natural genetic variations at the CETP locus can be used to help understand its impact on disease. Such human genetic studies have generated conflicting conclusions. The goal of the current investigation was to determine the association between CETP polymorphisms (I405V and TaqIB), and severity of coronary stenosis, since the extent of coronary artery narrowing has been considered as a primary determinant of survival in patients with coronary heart disease (CHD).

Methods

Study Population

The Onassis Cardiac Surgery Center ethics committee approved the protocol of this study. A total of 130 Caucasian Greek men with CHD were consecutively recruited among subjects admitted to our hospital for coronary angiography. The Onassis Cardiac Surgery Center is a major referral hospital for cardiac disorders; these patients were from various parts of Greece. CHD risk factors were also evaluated. According to the National Cholesterol Education Program - Adult Treatment Panel III guidelines¹⁵, diabetes mellitus was defined as fasting glucose > 126 mg/dl (7 mmol/L) or currently receiving antidiabetic medication; hypercholesterolemia was defined as total cholesterol > 170

mg/dl (4.4 mmol/L); hypertension was defined as $> 140/90$ mm Hg or currently on antihypertensive medication. All the patients recruited in the study gave informed consent.

Coronary angiography

Coronary angiography was performed by the Seldinger technique. The severity of CHD was defined by both Gensini score (GS)¹⁶ and Duke Jeopardy score (JS).¹⁷ The angiograms were examined by a qualified cardiologist. The GS and JS, measures of myocardial ischaemia extension, were computed by assigning a severity score to each coronary stenosis, according to the degree of luminal narrowing and its geographic importance.

Gensini score

Reduction in the diameter of the lumen and the X-ray appearance of concentric lesions as well as eccentric plaques were evaluated (reduction of 25%, 50%, 75%, 90%, 99%, and complete occlusion values were given GS of 1, 2, 4, 8, 16, and 32, respectively). To each principal vascular segment a multiplier, according to the functional importance of the myocardial area supplied by this segment was assigned: the left main coronary artery, x 5; the proximal segment of the left anterior descending coronary artery, x 2.5; the proximal segment of the circumflex artery, x 2.5, the mid-segment of the left anterior descending, x 1.5, the right coronary artery, the distal segment of the left anterior descending coronary artery, the posterolateral artery, and the obtuse marginal artery, x 1; and others, x 0.5.¹⁶

Duke Jeopardy score

The JS was developed by Dash et al¹⁷ and validated by Califf et al.¹⁸ The coronary tree is divided into six segments: the left anterior descending coronary artery, diagonal branches of the left anterior descending, septal perforating branches, the circumflex coronary artery, obtuse marginal branches, and the posterior descending coronary artery. All segments distal to 70% stenosis are considered to be at risk. Each such segment is assigned 2 points. The maximum possible number of points is 12.

CETP Genotyping

After the recruitment of the study population, genotyping of CETP polymorphisms (TaqIB and I405V) was performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis as described previously by others.^{19,20} Briefly, each PCR

reaction was performed using 500 ng of genomic DNA in a volume of 25 µl containing 50 mM KCl, 10 mM TRIS HCl (pH 8.8), 200 µM dNTPs, 1.0-1.5 mM MgCl₂, 12.5-25 pmol of each primer and 0.75 U of Taq polymerase (Keymed S.r.l., Rome, Italy). The intron 1 region containing the TaqIB polymorphism was amplified using the forward oligo 5'-CAC TAG CCC AGA GAG GGA GTG CC-3' and the reverse oligo 5'-CTG AGC CCA GCC GCA CAC TAA C-3', giving a fragment of 535 bp length.¹⁹ The exon 14 region containing the I405V polymorphism was amplified using the forward oligo 5'-TAT TTT TTT CAC GGA TGG GCA-3' and the reverse oligo 5'-TTG ACT GCA GGA AGC TCT GGC-3', giving a fragment of 142 bp length.²⁰ For the TaqIB polymorphism, the PCR conditions were an initial denaturation at 95°C for 6 min, followed by 30 cycles at 95°C for 30 sec, 65°C for 30 sec and 72°C for 30 sec and finally at 72°C for 5 min. For the I405V polymorphism, the PCR conditions were 95°C for 5 min, 60°C for 1 min and 72°C for 1 min for one cycle, and subsequently 35 cycles at 95°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec and finally at 72°C for 5 min.

For the detection of TaqIB polymorphism 5 µl of the PCR product were digested with 5 U of TaqI (New England Biolabs, Frankfurt, Germany) at 65°C overnight, giving 174 bp and 361 bp fragments in presence of the TaqI site. For the detection of I405V polymorphism 8 µl of the PCR product were digested with 5 U of MspI (New England Biolabs, Frankfurt, Germany) at 37°C overnight, giving 121 bp and 21 bp fragments in presence of the less common V allele.

Biochemical Analysis

Plasma total cholesterol, triglycerides and HDL-C were measured using enzymatic colorimetric methods on a Roche Integra Biochemical analyzer with commercially available kits (Roche Diagnostics GmbH, Mannheim, Germany). The serum LDL cholesterol levels were calculated using the Friedewald formula²¹ only in patients with triglyceride levels < 400 mg/dl (< 4.5 mmol/L). Lipoprotein (a) was measured by nephelometry (Nephelometer: BN-100, Behring, Germany). Blood glucose was measured by the hexokinase method with a Dade Behring reagent on a Dimension (Dade Behring) instrument. All samples were analyzed within 24 h.

Statistical Analysis

The results are given as the mean ± standard deviation (SD) unless otherwise stated. Differences in lipid lev-

TABLE 1. Characteristics of the study population.

Number of patients	130
Age (yr)	61(10)
Body mass index (kg/m ²)	27(4)
Total cholesterol (mg/dl)	229(53)
Triglycerides (mg/dl)	166(94)
HDL cholesterol (mg/dl)	38(10)
LDL cholesterol (mg/dl)	157(49)
Apolipoprotein A (mg/dl)	115(24)
Apolipoprotein B (mg/dl)	96(34)
Smoking (%)	41
Diabetes (%)	32
Hypercholesterolemia (%)	88
Hypertension (%)	69
Number of arteries stenosed	2(1)
Gensini score	100(105)
Duke Jeopardy score	6.4(4.4)
I allele frequency	0.68
V allele frequency	0.32
B1 allele frequency	0.60
B2 allele frequency	0.40

All values are presented as means (±standard deviation) or percentages.

HDL: high density lipoprotein, LDL: low density lipoprotein.

To convert total cholesterol, HDL and LDL concentration from mg/dl to mmol/L divide by 38.7. To convert TG concentration from mg/dl to mmol/L divide by 88.6.

els for the various genotypes were evaluated with one-way ANOVA and, if significant differences were found, by post hoc multiple comparisons. Differences in GS and JS between the CETP genotypes and between carriers of CETP alleles were analyzed by the unpaired t test, whereas ANOVA was utilised to compare all values among the CETP genotypes and carriers of CETP alleles. Correlations between GS or JS of subjects with different CETP genotypes were performed by Pearson two-tailed test. A value of P<0.05 was considered statistically significant.

Results

Characteristics of the study population.

Clinical characteristics of the study population and allele frequencies for the two CETP polymorphisms are shown in Table 1. The allele frequencies for both TaqIB and I405V are in Hardy-Weinberg equilibrium (table 1).

Differences in GS and JS according to CETP polymorphisms.

Homozygotes for the I allele displayed a lower GS compared with heterozygotes (IV) and with homozy-

TABLE 2. Distribution of CETP I405V (II, IV, VV) and TaqIB (B1B1, B1B2, B2B2) genotypes.

CETP polymorphisms	N (%)	GS (95% CI)	JS (95% CI)
I405V			
II	63 (49)	72(51-92)*	6.3(4.9-7.7)
IV	50 (39)	122(88-157)	6.4(5.5-7.3)
VV	17 (13)	136(74-198)†	6.9(5.7-8.2)
TaqIB			
B1B1	46 (35)	82(53-111)	6.3(4.5-8.1)
B1B2	65 (50)	108(83-132)	6.7(5.9-7.5)
B2B2	19 (15)	117(43-192)	5.4(4.0-6.8)

CETP: cholesteryl ester transfer protein, N: number of patients, GS: Gensini Score, JS: Duke Jeopardy score.

ANOVA test among I405V genotypes for GS, P=0.012.

*P=0.009 (unpaired t-test) between II and IV genotypes.

†P=0.010 (unpaired t-test) between II and VV genotypes.

TABLE 3. Blood lipid levels according to CETP polymorphism in all genotypes.

	Genotype	Mean	SD	P*
Total cholesterol (mg/dl)	II	230	50	0.536
	IV	232	55	
	VV	215	58	
	B1B1	232	45	
	B1B2	231	59	
	B2B2	216	51	
HDL-cholesterol (mg/dl)	II	36	9	0.15
	IV	40	11	
	VV	40	9	
	B1B1	37	9	
	B1B2	37	9	
	B2B2	42	14	
LDL-cholesterol (mg/dl)	II	158	45	0.694
	IV	159	52	
	VV	146	52	
	B1B1	162	40	
	B1B2	159	54	
	B2B2	142	48	
Triglycerides (mg/dl)	II	172	100	0.710
	IV	157	68	
	VV	174	133	
	B1B1	172	108	
	B1B2	166	90	
	B2B2	155	75	

HDL: high density lipoprotein, LDL: low density lipoprotein.

*P values among genotypes from Anova test performance.

To convert TC, HDL and LDL concentration from mg/dl to mmol/L divide by 38.7.

To convert TG concentration from mg/dl to mmol/L divide by 88.6.

gotes for V (Table 2). The GS score was also lower in carriers of I compared with carriers of V allele [86(95%CI 72-101) vs. 128(95%CI 102-154), P=0.003]. In contrast, the GS did not differ in subjects with the various TaqIB genotypes, neither in B1 and B2 allele carriers (Table 2). The JS was not influenced by neither CETP polymorphism (Table 2).

Differences in lipid profiles according to CETP polymorphisms.

The lipid profile was not significantly affected neither by the I405V nor the TaqIB polymorphism (Table 3).

Correlations

A correlation was detected between the I405V polymorphism and the GS (Pearson 2-tailed test) $r=0.250$, P=0.005. On the other hand, no correlation was found between JS and any of the CETP polymorphisms.

Discussion

The current study first investigated the correlation of genetic variations in the CETP gene and severity of coronary stenosis estimated by GS and JS among Greek population. Compared with simple considerations of the number of diseased vessels, the GS and JS allow variability in importance of each of the three major coronary arteries in each patient. Our data showed that the presence of the I allele is linked with less severe coronary stenosis estimated by the GS.

The frequencies of CETP I405V and TaqIB gene polymorphisms were also determined in this population. The V405 allele appeared at a frequency of 0.421 and 0.440 in CHD Han Chinese patients and controls, respectively²² similar to its frequency in Caucasians,⁹ but lower than its frequency in Japanese-Americans from Honolulu²³ and higher than in our population (0.32). The TaqI-B2 allele frequency was 0.394 in Han Chinese CHD patients and 0.395 in Han Chinese controls, slightly lower than in Caucasians^{9,24} but close to its frequency in Japanese²³ and close to our patients (0.40).

Gene CETP variations have a strong impact on CETP activity and thus on HDL-C levels.²⁰ Although allele frequencies vary among different populations, many previous studies have shown that the TaqI-B2 allele of the CETP gene is associated with higher plasma HDL-C levels in Caucasians.^{9,23,24} In contrast, Arca et al found no association between TaqI-B2 and HDL-C level in a group of 415 subjects, with angiographically documented CHD, in Italy.²⁵ Still, other

investigators have reported that the TaqI-B2 allele is not associated with increased HDL-C levels in Italian migrants to Australia²⁶ and in healthy African Americans.²⁷ The results of studies reporting no association of TaqIB polymorphism with HDL-C levels are in accordance with our findings in the Greek population. A possible explanation for this diversity among populations may be differences in the criteria for subject enrolment and differences in composition of the study groups. More importantly, the diversity may account for differences in the genetic background of the various populations, which may interact with environmental factors, such as differences in alcohol consumption and tobacco smoking.^{9,28} For example, approximately 50% of Greek population are smokers.²⁹

No differences were observed in lipid profiles of subjects with various genotypes of both CETP polymorphisms. Similar results were observed after studying I/V and B1/B2 carriers. Thus, the combination of multiple studies allows one to confirm this phenotype/genotype association more rigorously.

Nevertheless, our data revealed that homozygotes and carriers of the I, compared with the V, allele may display lower severity of coronary stenosis, estimated by GS. As far as it concerns the JS, no differences were found in subjects with different I405V or TaqIB genotypes. This may be attributed to the fact that the severity of stenosis can be estimated more accurately by the GS, in comparison with JS. The larger range of GS allows greater variability in estimation depending on the degree and the area of the stenosis. The few patients who were genotyped limited the power of the study. Additionally, the alcohol status of our study population was undefined.

Our findings indicate that the I405V polymorphism may have potential importance in screening individuals at high risk for developing CHD, establishing efficient prevention measures, and searching for other risk factors for CHD. It seems that the I405V CETP polymorphism may influence the severity of coronary stenosis. In order to define such conclusion, larger studies in various populations are needed.

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ORIGINAL RESEARCH

Exercise Training and Heart Rate Variability in Older Adult Female Subjects

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The results of this paper have been presented previously at the Canadian Society of Geriatric Medicine and the American Gerontological Association Meetings.

The Effects of Endurance and Strength Training on Measures of Heart Rate Variability in Healthy Elderly Female Subjects

INTRODUCTION: Prior investigations in post-myocardial infarction and healthy elderly subjects have established that heart rate variability (HRV) predicts mortality. Predominantly cross-sectional studies have shown an association between endurance training and measures of HRV. In a randomized trial, this study sought to prospectively compare the effects of endurance and strength training on HRV in 45 healthy elderly females (average age 69.9 ± 0.9 years).

METHODS: All subjects were rigorously screened to be normal by history, physical, blood tests, ECG, ETT and echocardiogram. All subjects were monitored for 24 hours by a 2-channel Holter before and after training. Artifacts and arrhythmias were manually removed. Tapes were examined for standard measures of HRV.

INTERVENTION: 15 subjects were randomized to endurance trained (ET), 15 subjects to strength training (ST), and 15 subjects to no training (NT) for six months. **RESULTS:** Training resulted in a significant increase in VO_{2max} in the ET (+7.4%, $p=0.005$) group only. There was a small but not significant decrease in HR with both the ET and ST groups. ET resulted in a significant increase in most time domain and all frequency domain measures of HRV. ST resulted in no significant change in HRV measures.

CONCLUSION: Strength training, as opposed to endurance training has no significant impact on HRV. This suggests that exercise interventions designed to improve strength (such as weight-lifting) will have little to no impact on HRV, suggesting that aerobic and strength training operate through different mechanisms to reduce cardiac risk.

ABSTRACT WORD COUNT:

Key Words: aerobic training, resistance training, autonomic, geriatric, cardiovascular risk

INTRODUCTION

Both aerobic and strength training have been shown to significantly impact risk factors for cardiovascular disease. The benefits of increased aerobic activity in the older population include loss of body fat, reduced rate of development of diabetes, reduction of hypertension, improved lipid profiles, increased maximal cardiac output, and increased maximal oxygen consumption(1). Strength training has been shown to increase lean body mass(2), reduce abdominal girth, increase treadmill endurance(3, 4), increase maximal walking endurance(5, 6), and lower resting blood pressure(7-10). Although all these factors are recognized predictors of cardiovascular mortality, the effects of aerobic and strength training on cardiac autonomic nervous system function are poorly studied in the older adult population. One measure with the potential to provide an explanation for the effects of different training modalities on cardiovascular mortality is heart rate variability (HRV).

A reduction in time and frequency domain measures of heart rate variability (HRV) have been shown to be independent predictors of mortality in post-myocardial infarction patients(11-13), patients at risk of sudden death(14), heart failure(15), and diabetes mellitus(16) and can also predict mortality in healthy middle-aged and elderly persons(17,18). This suggests that measures of HRV could prove valuable in predicting future cardiac events in the elderly population, and that interventions (such as exercise) that are thought to improve HRV might reduce the risk of such events.

Despite the fact that HRV has been shown to decrease as a function of age(19), there is poor understanding of how different forms of exercise (such as aerobic and resistive training) affect HRV in the elderly population. In older populations, the effects of aerobic training have yielded conflicting results, likely due to different screening criteria and the level of rigor with which the exercise protocols were monitored. Studies of aerobic training in older men have shown either no HRV training effect(20) or a large training effect (21); studies of a mixed group of older men and women showed a training effect during daytime HRV measures only (22). Strength training interventions in older healthy adults have either shown no effect (23) or an increase in the high frequency component (0.15-0.4 Hz) of HRV(8) only. Clearly a prospective, randomized trial of aerobic and strength training is needed to examine the impact of different forms of training on cardiac autonomic function.

The purpose of this study was to prospectively examine the effects of endurance and resistive training in an older population of normal, healthy women in a controlled, randomized fashion. Our hypothesis was that aerobic training would have a beneficial effect, while strength training would have no effect on HRV in this population.

METHODS

Subjects

The subjects consisted of 50 older (aged 60 to 79 years) healthy female adults, recruited from the local community through advertisement in local publications. 15 subjects were randomized to each of three groups: a sedentary group (NT), an endurance trained group (ET), and a strength training group (ST). Subjects were excluded if they had any history of angina, myocardial infarction, stroke, hypertension, chronic pulmonary disease, diabetes, current medication use (prescription or over the counter), current smoking, or exercise-limiting orthopedic impairment. Entry requirements included a normal blood pressure, a normal physical exam, normal resting ECG, normal M-mode and two-dimensional echocardiograms showing no more than mild valvular regurgitation, a normal Bruce protocol treadmill maximal exercise stress test, and a normal hematocrit, fasting blood glucose, total cholesterol, and creatinine. 5 subjects were excluded on the basis of this screening, leaving a total of 45 subjects participating in the study (15 randomized to each group).

This study was approved by the Human Subjects Committee of the University of Washington, and all subjects gave written informed consent.

Training Program

The endurance training intervention was designed to improve aerobic fitness according to current guidelines(1, 24), and consisted of moderate to vigorous intensity exercise on a cycle ergometer. Training sessions were five times per week, and subjects had to attend 90% of all training sessions to remain enrolled in the study. Target heart rates for the ET group were based on maximal HR observed during maximal exercise treadmill testing (see below). The endurance training program used a target heart rate zone of 50 to 60% of maximal heart rate for the first two months, progressing to 80 to 85% of maximal heart rate for the remainder of the program. Heart rates were monitored at 60-second intervals during training using Polar Vantage Heart Watches.

The ST group underwent a program of weight training designed to improve strength according to current guidelines(1, 24, 25). Sessions were five times per week and were supervised by a certified trainer. The program consisted of 10 exercises designed to improve both upper and lower body strength and utilized free weights and commercially available equipment. The goal was to progress to 85% of a one-repetition maximum (which was determined for each muscle group initially and at four-week intervals). Training consisted of 3 sets of 8 to 12 repetitions per set, for each muscle group.

Data Collection and Processing

Maximal oxygen consumption and maximal HR were determined using a maximal Bruce treadmill protocol exercise test. Changes in maximal oxygen consumption were examined in all groups, including the untrained and strength trained subjects. The mean expiratory exchange ratio was 1.13 ± 0.02 on the tests before training and 1.16 ± 0.02 on the tests after training, indicating good effort. Submaximal HR and submaximal oxygen consumption was recorded at a common workload (2.5 mph, at a 12% Grade) for each subject.

All subjects received 24-hour monitoring by a 2-channel Holter (Spacelabs) before and after the 6-month training protocol. Each tape was manually examined using a Spacelabs FT2000 workstation, which converted ECG data from analog to digital at a sampling rate of 500 Hz. Each 24-hour data collection was manually examined in 15 minute segments to ensure that each segment was free of artifacts or arrhythmias. 8 tapes (1 NT, 6 ET and 1 ST) had to be discarded due to the presence of excessive PACs or low voltage readings. Data collection met the recording requirements for long-term recordings as outlined by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.(13)

After the 24-hour data was converted to digital form and edited, the data was analyzed for previously well-established measures of HRV in both the time and frequency domains(13). In the time domain, 24-hour data was examined for mean heart rate (HR), standard deviation (SDNN), standard deviation of 5-minute mean RR-intervals (SDANN), mean of all 5-minute standard deviations of RR-intervals (SD), root-mean square of difference of successive RR intervals (rMSSD) and the proportion of adjacent RR-intervals more than 50 msec different (%RR50).

In the frequency domain, a commercially available

software program (Spacelabs) was used to determine low frequency (LF, 0.04-0.15 Hz), high frequency (HF, 0.15 to 0.4 Hz) and total frequency power (TP), using a commercial program (Spacelabs). This software divides the digitally scanned data into 4-minute segments and performs a Fast Fourier Transform on each segment. A mean LF, HF and TP is then calculated for 24 hours, daytime (09:00 to 15:00) and nighttime (00:00 to 06:00).

Statistical Analysis

The effects of training on measures of HRV and measures of physical fitness were calculated by a one-way analysis of variance for repeated measures(26). The effects of training on circadian changes in HRV measures were examined by comparing the delta value (the value from 00:00 to 06:00 minus the value from 09:00 to 15:00) before and after training using a paired t-test(26).

RESULTS

Baseline Measurements

At baseline, there was no significant difference between the three exercise groups in terms of age, weight, height, body mass index, resting heart rate, supine blood pressure or maximal volume of oxygen uptake (VO_{2max}) (Table 1).

Effects of Exercise Training on Levels of Fitness:

A significant increase in VO_{2max} was seen in the ET group ($+7.4 \pm 2.5\%$, $p=0.005$) while VO_{2max} showed a significant decrease in the NT group. There was no significant change in VO_{2max} in the ST group, indicating little change in aerobic fitness with strength training. The different exercise interventions had no effect on maximal HR. Both ST and ET decreased both the heart rate and VO_2 found at a submaximal work load (at 2.5 mph at a 12% grade) (Table 2).

Baseline Measures of HRV

There was no significant difference between the three groups with respect to baseline HR, time domain measures of HRV (%RR50, RMSSD, SD, SDANN, SDNN) or frequency domain measures of HRV (HF, MF, TP) (Tables 3 and 4).

Untrained Group

The NT group showed no change in HR, time domain measures of HRV (%RR50, RMSSD, SD, SDANN, SDNN) or frequency domain measures of HRV (HF, MF or TP) with no training (Tables 3 and 4).

TABLE 1-Baseline Subject Characteristics

Mean ± SEM	NT (n=15)	ET (n=10)	ST (n=15)	P value (Effect of Group)
Age (years)	71.8±1.2	70.0±2.6	69.8±1.5	0.63
Weight (kg)	74.2±3.1	71.5±6.1	72.8±5.0	0.92
Height (cm)	162.4±1.3	158.5±2.1	164.4±2.7	0.20
Body Surface Area (m ²)	1.80±0.04	1.73±0.07	1.82±0.07	0.61
Body Mass Index (kg/m ²)	28.2±1.3	28.5±2.2	26.8±1.5	0.75
Maximal Volume of Oxygen Uptake (VO _{2max} , mL/kg/min)	20.6±0.9	23.0±1.6	20.7±1.0	0.30
Maximal HR (bpm)	156.6±2.8	157.0±2.9	156.4±4.3	0.99
Submaximal VO ₂ (2.5 mph, 12% Grade) (mL/kg/min)	15.2±0.7	15.3±0.7	15.1±0.6	0.98
Submaximal HR (2.5 mph at 12% Grade) (beats/min)	125.8±4.2	124.3±5.3	125.6±3.5	0.97

Table 1: Baseline subject characteristics for the untrained (NT), strength trained (ST) and endurance trained (ET) groups. The P value reflects the effect of exercise group on baseline measures. Units of variation are presented as plus or minus standard error.

TABLE 2-Training Effects on Measures of Fitness

Delta Values	NT (n=15)	ET (n=10)	ST (n=15)	Analysis of Variance, Training Effect, P Values		
				NT	ET	ST
VO _{2max}	-0.6±0.3	+1.5±0.5	+0.2±0.5	0.03*	0.005*	0.39
	-3.0±1.4%	+7.4±2.5%	+1.6±2.4%			
HR _{max}	+1.5±1.7	+0.8±1.2	+1.6±2.1	0.41	0.55	0.45
	+1.0±1.1%	+0.5±0.3%	+1.0±1.3%			
VO _{2 submax}	-0.4±0.6	-1.4±0.5	-1.6±0.4	0.57	0.03*	0.004*
	-1.7±3.5%	-8.9±3.0%	-10.6±2.8%			
HR _{submax}	+6.8±2.1	-10.4±3.0	-10.5±3.5	0.008*	0.010*	0.013*
	+5.4±1.7%	-8.4±2.4%	-8.4±2.8%			

Table 2: Absolute and relative effects of six months of no training (NT), endurance training (ET) or strength training (ST) on markers of physical fitness on maximal volume of oxygen uptake (VO_{2max}), maximal heart rate (HR_{max}), submaximal volume of oxygen uptake (VO_{2 submax}) and submaximal heart rate (HR_{submax}). All submaximal measures were taken at a speed of 2.5 miles per hour at a 12% grade. The symbol * designates a significant training effect (using Analysis of Variance, p<0.05) between different training groups. Units of variation are presented as plus or minus standard error.

Effects of Endurance Training on Measures of HRV

There was a non-significant trend for resting heart rate to decrease with 6 months of endurance training (p=0.13). Endurance exercise training resulted in a significant increase in several measures of HRV. Overall cumulative variability in HR, as measured by standard deviation, showed a significant training effect in the ET group (+16%, p=0.02). %RR50 (a measure of short-term HRV) had a nonsignificant trend towards a training-induced increase. SDANN, which is influenced by long-term variations in HR (longer than 5 minutes) increased (+21%, p=0.02) with endurance training. RMSSD and SD showed no significant training effect (Table 3).

In the frequency domain, the ET group demonstrated a training-induced increase in low frequency power (+81%, p=0.004), high frequency power

(+48%, p=0.046), and total power (+50%, p=0.046) (Table 4).

Effects of Strength Training on Measures of HRV

6 months of strength training resulted in no significant change in resting heart rate or in any HRV parameters (Tables 3 and 4).

Circadian Rhythm in HRV parameters

The magnitude of circadian rhythms in HRV (the "circadian delta" value) was determined by taking the nighttime value (00:00 to 06:00) and subtracting the daytime value (09:00 to 15:00). All subjects at baseline showed a significant elevation in nighttime versus daytime measures of %RR50 (p<0.0001), RR50 (p=0.001), RMSSD (p=0.04), SD (0.006), SDANN (p<0.0001), SDNN (p<0.0001), HF (p<0.0001), LF

TABLE 3-Effects of Training on Time Domain Measures of Heart Rate Variability

		<i>NT</i> (<i>n=14</i>)	<i>ET</i> (<i>n=9</i>)	<i>ST</i> (<i>n=14</i>)	<i>Analysis of Variance, Training Effect, P Values</i>		
					<i>NT</i>	<i>ET</i>	<i>ST</i>
HR (bpm)	Baseline	72.5±1.4	76.3±2.2	73.8±1.7	0.29	0.13	0.80
	Delta	-1±1	-3±2	-2±1			
%RR50 (%)	Baseline	5.4±2.0	2.4±0.8	4.9±1.3	0.47	0.10	0.58
	Delta	-0.5±0.6	+3.3±1.8	+2.0±0.5			
RMSSD (ms)	Baseline	37.6±4.6	29.9±4.7	34.2±2.8	0.76	0.50	0.96
	Delta	-1.1±3.8	+2.1±2.9	+1.9±2.4			
SD (ms)	Baseline	49.0±4.0	44.4±4.7	46.5±3.0	0.64	0.28	0.56
	Delta	-2.0±2.2	+4.0±3.5	+2.4±1.6			
SDANN (ms)	Baseline	110.3±6.1	93.0±5.5	112.0±4.9	0.36	0.02*	0.61
	Delta	-3.2±5.9	+19.3±7.0	-10.1±5.6			
SDNN (ms)	Baseline	124.2±6.5	107.1±5.6	123.7±5.3	0.19	0.02*	0.61
	Delta	-5.6±5.0	+16.7±5.8	-8.4±5.7			

Table 3: This table depicts the effects of six months of strength (ST), endurance (ET) or no training (NT) on heart rate (HR), proportion of adjacent RR-intervals more than 50 msec different (%RR50), mean of all 5-minute standard deviations of RR-intervals (SD), standard deviation of 5-minute mean RR-intervals (SDANN), and standard deviation (SDNN). "Delta" represents the change in each HRV from baseline after 6 months of the different training regimes. Units of variation are presented as plus or minus standard error.

The symbol * designates a significant training effect (using analysis of variance, $p < 0.05$) between different training groups.

TABLE 4-Effects of Training on Frequency Domain Measures of Heart Rate Variability

		<i>NT</i> (<i>n=14</i>)	<i>ET</i> (<i>n=9</i>)	<i>ST</i> (<i>n=14</i>)	<i>Analysis of Variance, Training Effect, P Values</i>		
					<i>NT</i>	<i>ET</i>	<i>ST</i>
HP	Baseline	344±104	254±72	268±38	0.60	0.046*	0.11
	Delta	+11±129	+121±51	+51±30			
LP	Baseline	676±204	459±96	568±88	0.97	0.004*	0.30
	Delta	-209±139	+373±57	+208±94			
TP	Baseline	2470±535	1749±293	2162±253	0.72	<0.001*	0.05
	Delta	-314±446	+870±121	+138±135			

Table 4: This table depicts the effects of six months of strength (ST), endurance (ET) or no training (NT) on high frequency power (0.15 to 0.40 Hz, HP), low frequency power (0.04 to 0.15 Hz, LP) and total power (0.04 to 0.15 Hz, TP). All measures of power are in ms^2 . "Delta" represents the change in each HRV from baseline after 6 months of the different training regimes. Units of variation are presented as plus or minus standard error.

The symbol * designates a significant training effect (using analysis of variance, $p < 0.05$) between different training groups.

($p < 0.0001$), and TP ($p < 0.0001$). Heart rate was also significantly slower at nighttime ($p < 0.0001$). There was no training effect on any of these circadian rhythms.

DISCUSSION

Endurance training significantly increased HRV in both time (SDANN and standard deviation) and fre-

quency domains. Strength training, however, had no effect on measures of HRV as compared to the untrained control group. This confirms our hypothesis that endurance training can have a positive impact on HRV in older adult women, while strength training does not. This study is unique in that it is the first to rigorously compare in a randomized controlled fashion the effects of a long-term endurance and

strength training interventions on HRV in a group of older women.

Previous studies have examined the effects of endurance training in older subjects, but the study populations were either mixed or all male(20-22, 27). A nine-month exercise intervention in older males increased the 24-hour Holter measure of standard deviation by 12.7% (which agrees with the 16% increase seen in the present study)(20). A six-month endurance training intervention increased standard deviation by 68%(21), although that study used 5 minutes of heart rate data only and the magnitude of the increase cannot be compared with our 24-hour Holter data(13, 19). Contrary to the above results, another six-month endurance training intervention by Schuit et al. (in a group of mixed gender) had no effect on 24-hour Holter measures of HRV and a 6% increase in standard deviation (+6%) when only daytime data was considered(22). The effect of endurance training in the study by Schuit et al. was diluted by the fact that the subjects at the start of the study were a mix of sedentary and nonsedentary subjects; about half of the study participants already considered themselves "active" prior to the start of the exercise intervention(22). Our results are concordant with previous studies involving middle aged subjects(28) and young males(29).

Our study results showing no effect of strength training on HRV were concordant with the effects of 16 weeks of dynamic resistance training in healthy older women using a cycle ergometer set to high resistance(23). However, Taylor et al. found an increase in high frequency power in older hypertensives with isometric handgrip training (8) which was not found in the present study. One explanation for this is that Taylor et al. used isometric handgrip training to avoid increases in blood pressure during training in a hypertensive population(8). Both our study and the high resistance cycle ergometer study(23) studied subjects without hypertension, which allowed us to train large muscle groups according to current guidelines(1, 24, 25). Strength training of large muscle groups, unlike isometric handgrip training, produces large swings in blood pressure (about 50 mm of Hg increase in systolic blood pressure during a 50% maximal contraction in older adults)(30). Taylor et al. postulated that strength training resulted in a central resetting of the arterial baroreceptor reflex through an as yet unknown mechanism which would result in an increase in vagally-mediated high frequency oscillations in heart rate(8). But recent literature in other

subject populations has suggested that strength training of large muscle groups increases arterial stiffness(31, 32). An increase in arterial stiffness would be expected to reduce arterial baroreceptor sensitivity since the arterial baroreceptors are located in the aortic and carotid arterial walls(33). A reduction in arterial baroreceptor sensitivity results in a reduction in all measures of heart rate variability(34-36) (including high frequency power) which would obscure the increase in vagally-mediated high frequency power seen by Taylor et al. No measures of arterial stiffness were obtained in either the investigation by Taylor et al or the present study, which makes this explanation purely speculative.

Postulated Mechanisms

Several mechanisms for the differential effect of endurance and strength training can be postulated from the frequency domain measures of HRV. Some previous investigations have suggested that endurance training increases vagal activity(37). Since high frequency oscillations appear to be mediated primarily by changes in vagal activity(38, 39), an increase in vagal activity with endurance training is supported in the present study; the ET group showed a significant increase in high frequency power while the ST group did not. Training-induced bradycardia in dogs was prevented by cardiac-denervation, suggesting that endurance training affects HRV by an increase in vagal tone(40). Endurance training in young human subjects have also shown an increase in the cardiovagal phase of the Valsalva maneuver(41) and in the vagal reactivation seen in the recovery phase of acute exercise(42).

Strength training, however, appeared to have little effect on vagal modulation of heart rate, as shown in the lack of a significant increase in high frequency power. The normal process of aging is known to result in increased arterial stiffness due to an increase in intima and media thickness, smooth muscle cell hyperplasia, and extracellular matrix proliferation(43). Increased arterial stiffness dampens the arterial baroreflex due to the fact that these receptors are located in both the carotid and aortic arterial walls(33). Numerous cross-sectional and prospective studies have shown that aerobic exercise improves arterial compliance (44-47) while strength training decreases arterial compliance(31,32). It has been hypothesized that the increased pulse pressures and mechanical distension during aerobic exercise sessions result in pulsatile "stretching" of the collagen fibers

that reverse the age associated reduction in arterial compliance(48). However, strength training involves abrupt and sustained elevations in blood pressure that result in stiffening of the large vessels which would dampen the arterial baroreflex(31). Increased arterial compliance has been associated with an increased HRV (through an increase in arterial baroreceptor sensitivity) and this could explain why endurance training increased HRV while strength training had no impact on HRV(36).

Circadian Rhythms:

It has been well established that HRV increases in the early morning hours(19) in healthy younger subjects. This is the first observation, to our knowledge, that the circadian rhythm in HRV remains intact in healthy older adult women. Exercise training (either endurance or strength training) had no impact on the magnitude of this circadian rhythm.

Clinical Implications:

Measures of HRV have been shown to be independent predictors of mortality in the older adult population(17, 18), suggesting that any intervention that improves these measures might be protective against sudden cardiac death. Although both endurance and strength training have been shown to modify other risk factors for cardiac death, only endurance training seems to have an impact via an increase in HRV. This is not to suggest that strength training has no value in preventing cardiac death in older adult women, but the results of this study show that aerobic and strength training are not interchangeable in terms of reducing cardiac risk. Only aerobic training reduces cardiac risk through an increase in HRV, indicating that aerobic and strength training should be used to complement each other.

Limitations:

Caution must be used in making mechanistic or clinical conclusions from HRV data. HRV is significantly related to vagal tone at the sinus node, but is not a direct marker of vagal activity(49, 50). Further work is required to examine the differential physiological mechanisms responsible for the effects of endurance and strength training on cardiovascular mortality.

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PRACTICE GUIDELINES

Canadian Consensus Guidelines for the Diagnosis and Management of Acromegaly

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Abstract

Acromegaly is a chronic condition associated with considerably increased morbidity and mortality if left unchecked. In December 2004, a national meeting was held to discuss the diversity in clinical practice across the country in diagnosing and treating patients with acromegaly, as well as to seek consensus on a number of management principles. The group reviewed recent guidelines and discussed issues of diagnosis, treatment, monitoring and treating comorbidities to seek a Canadian consensus on the management of this rare disorder.

Consensus was that diagnosis should include clinical and biochemical findings, but is hinged on establishing GH hypersecretion with IGF-I and OGTT testing. Treatment has traditionally included surgical resection or debulking, along with adjunctive medical

therapy (primarily somatostatin analogues), if necessary, to normalize GH levels. The option of primary medical therapy in managing this condition has recently emerged and can be justified for non-surgical candidates or for those in whom surgery is not expected to be curative. Overall, improved screening practices and superior epidemiological data are required, since timely diagnosis and appropriate treatment are crucial for reducing the potentially debilitating effects of this chronic, progressive disease. The current evidence also supports the need for long-term follow-up of disease activity and comorbidities in diagnosed patients.

A national meeting was held to discuss the diversity in clinical practice across the country in diagnosing and treating patients with acromegaly, as well as to seek consensus on a number of management principles. After brief reviews of the most recent Canadian guidelines and the 2004 guidelines published by the American Association of Clinical Endocrinologists, the group was asked to specifically examine the issues of diagnosis, treatment, monitoring and treating comorbidities and seek a Canadian consensus on prac-

tice. This paper summarizes the working group's findings and the points of consensus that were achieved.

Introduction

Acromegaly is a relatively rare disorder characterized by the hypersecretion of growth hormone (GH), usually the result of a pituitary somatotroph adenoma. GH stimulates production of insulin-like growth factor-I (IGF-I), the levels of which are also elevated in acromegaly. Its clinical presentation in advanced stages may include coarsened facial features, protruding jaw, widely spaced teeth, and large hands and feet.^{2,3} Patients may complain of headache and/or fatigue. Other manifestations may include unexplained hypertension, cardiovascular disease, diabetes mellitus, sleep apnea, hyperhidrosis, hypogonadism, carpal tunnel syndrome, and/or hypertrophic arthropathy.⁴ Symptoms of tumour growth may include headache, visual field defects, and/or cranial nerve entrapment.^{2,3}

Acromegaly is a chronic, progressive, potentially debilitating condition associated with significant morbidity and increased mortality. The mortality rate for uncontrolled disease activity is 2-4 times higher than that of the general population, principally because of the increased risk of cardiovascular disease.^{2,5,6} This increased mortality can be reversed if the elevated GH and IGF-I levels are successfully normalized.⁷⁻¹¹ The increased morbidity is due to the metabolic effects of elevated GH and IGF-I, and to the mass effects of the pituitary adenoma. Depending on the comorbid condition, effects may also be partly reversible with normalization of hormone levels. For example, soft-tissue overgrowth can be at least partly diminished, but correcting bone enlargement may require surgical intervention.¹²

The estimated annual incidence, based on relatively old data, is 3-4 cases per million.^{13,14} This is probably an underestimate, but with no North American data, the true prevalence of the disease remains unclear. What is known is that, on average, those diagnosed with acromegaly have had the disease for nearly 10-15 years.¹⁵ As a result, the majority of patients are diagnosed at an advanced stage of their disease when their pituitary tumours are classified as macroadenomas (>10 mm in diameter).¹⁶ Improved screening and more accurate epidemiological data are needed to assess better the extent of the problem and improve diagnosis and treatment methods. Timely diagnosis and appropriate treatment are crucial to reducing the increased morbidity and mortality associated with this chronic, progressive disease.

With the publication of newer data and developments in available treatment modalities since the pub-

TABLE 1A. Quality of evidence¹

Diagnostic studies	
Level 1	<ul style="list-style-type: none"> i) Independent interpretation of test results (without knowledge of the result of the diagnostic or gold standard) ii) Independent interpretation of the diagnostic standard (without knowledge of the test result) iii) Selection of people suspected (but not known) to have the disorder iv) Reproducible description of both the test and diagnostic standard v) At least 50 patients with and 50 patients without the disorder
Level 2	Meets 4 of the Level 1 criteria
Level 3	Meets 3 of the Level 1 criteria
Level 4	Meets 1 or 2 of the Level 1 criteria
Therapeutic studies	
Level 1A	<ul style="list-style-type: none"> • Systematic overview or meta-analysis of high-quality randomized, controlled trials • Appropriately designed randomized, controlled trial with adequate power to answer the question posed by the investigators
Level 1B	Nonrandomized clinical trial or cohort study with indisputable results
Level 2	Randomized, controlled trial or systematic overview that does not meet Level 1 criteria
Level 3	Nonrandomized clinical trial or cohort study
Level 4	Other
Prognostic studies	
Level 1	<ul style="list-style-type: none"> a) Inception cohort of patients with the condition of interest, but free of the outcome of interest b) Reproducible inclusion/exclusion criteria c) Follow-up of at least 80% of subjects d) Statistical adjustment for extraneous prognostic factors (confounders) e) Reproducible description of outcome measures
Level 2	Meets criterion a) above, plus 3 of the other 4 criteria
Level 3	Meets criterion a) above, plus 2 of the other criteria
Level 4	Meets criterion a) above, plus 1 of the other criteria

TABLE 1B. Grades of recommendations¹

Grade	Criteria
Grade A	The best evidence was at Level 1
Grade B	The best evidence was at Level 2
Grade C	The best evidence was at Level 3
Grade D	The best evidence was at Level 4 or consensus

TABLE 2. Differences from the 2000 Canadian guidelines²

- Call for improved screening, more current North American epidemiological data.
- Definition of target populations for screening.
- Recommendation of IGF-I or OGTT (with GH) as screening test, depending on local availability and facilities.
- Recommendation of IGF-I and OGTT (with GH), plus clinical findings, for diagnosis.
- Two new treatment algorithms, including one devoted to medical therapy.
- Recommendation for decreased use of non-selective dopamine agonists; bromocriptine no longer recommended.
- Stronger recommendation for use of GH receptor antagonists (pegvisomant).
- Emerging support for primary medical therapy in patients who are not good surgical candidates or for whom surgery is not expected to be curative.
- Discussion of monitoring and treatment of related comorbidities.
- Recommendation to assess and stabilize comorbidities prior to treatment.

lication of the previous Canadian guidelines document,³ the need for an updated consensus document on the diagnosis and treatment of acromegaly was identified. The levels of evidence and grading of recommendations are shown in Tables 1.

This consensus document builds on those previous Canadian guidelines³ (Table 2) and includes new treatment algorithms and updated treatment recommendations.

This document also presents a Canadian alternative to the recently published U.S. guidelines,² taking into account local realities in its screening and diagnosis recommendations, providing treatment approaches based on the context and experience of Canadian experts (e.g., recommending addition or substitution of the dopamine agonist cabergoline prior to the use of a GH receptor antagonist), furnishing new treatment algorithms and pointing to Canadian guidelines for the monitoring and treatment of related comorbidities.

Screening and Diagnosis

Screening

Because acromegaly is slowly progressive, and because signs and symptoms are common and physical changes may initially be subtle, diagnosis has tended to occur late in the course of the disease.¹⁶ One study found the mean delay in diagnosis to be 9.2 yr.¹⁵ Such delays have serious repercussions. First, because acromegaly is such a disfiguring condition, by the time the diagnosis is

TABLE 3. Unexplained constellations of signs and symptoms

Unexplained constellation of common symptoms, including the following¹⁵:

- Sweating
- Headaches
- Fatigue
- Arthralgias
- Visual field impairment
- Hypogonadism
- Decreased energy
- Muscular weakness
- Depression
- Decreased libido
- Paresthesiae
- Carpal tunnel syndrome

Unexplained constellation of common signs, including the following¹⁵:

- Hypertension
- Sleep apnea
- Diabetes/impaired fasting glucose
- Peripheral neuropathy
- Osteoarthritis
- Carpal tunnel syndrome

made the patient's physical appearance has often been altered irreversibly, and the metabolic and cardiovascular effects have taken a toll on the patient's quality of life and potential survival. Second, diagnosing tumours when they have become macroadenomas reduces the chances for achieving strict control of the disease and increases the chances of damage to the remaining pituitary tissue with currently available therapies. These very important facts show the need for an increased awareness of the early manifestations of the disease among primary care providers, allowing for selective screening of individuals at risk.

Naturally indiscriminate screening of a large population would not be feasible or useful for what is still a relatively rare condition; carefully defining the population to be screened is essential in order to focus inquiry on those patients demonstrating evident signs and/or symptomatology. For this reason, it is recommended to screen a target population that may be at increased risk of harbouring the disease [Grade A, Level 1]. Such a population would include those with a constellation of the following apparently common but unexplained symptoms and signs, including the following: pituitary incidentaloma; recurrent colon polyps; sleep apnea; multiple skin tags; unexplained carpal tunnel syndrome; unexplained and persistent soft-tissue swelling, such as

hand/foot soft-tissue swelling, jaw enlargement and/or dental malocclusion; unexplained oligomenorrhea or amenorrhea; unexplained hypertrophic osteoarthritis; impaired glucose tolerance or diabetes mellitus particularly in the absence of a family history, and presenting in combination with any of the above. Constellations of such symptoms and signs are summarized in Table 3.

These features are particularly relevant in patients with acromegaly and should lead to consideration for screening.

Screening should be performed using a serum IGF-I level¹⁶ or a 75 g oral glucose tolerance test (OGTT) with GH levels, depending on local availability and facilities [Grade A, Level 1].

Diagnosis

A definitive diagnosis of acromegaly is ideally based on both biochemical and clinical findings. When there are sufficient clinical grounds to suspect a patient has acromegaly, laboratory testing should be performed to confirm elevation of growth hormone and establish a diagnosis. Both the IGF-I and the OGTT are preferred for the purposes of diagnosis: the IGF-I usually as an initial screening test, and the 2-hour OGTT for confirmation.

It should be noted that both IGF-I and OGTT can be used for screening and/or diagnosis, but each has its own limitations that must be taken into account when selecting a test. Nevertheless, testing for acromegaly must also be applicable and practical in the community, and a 2-hour specialized OGTT for growth hormone may not always be available in outreach centres, for example.

In situations in which there is discordance between clinical suspicions and biochemical findings, patients should be referred to an endocrine centre of excellence for further investigation [Grade D, consensus].

Clinical findings

Clinical suspicion of acromegaly should be raised when patients present with any of the signs and symptoms mentioned in Screening, above, but especially when they present with a combination of two or more of these findings: soft-tissue swelling, such as hand/foot soft-tissue swelling or enlargement; unexplained carpal tunnel syndrome or other nerve-entrapment syndromes; jaw enlargement and/or dental malocclusion; visual field defects; unexplained and atypical headaches; scalp and forehead skin folding or increase in hat size; nasal polyps; obstructive sleep apnea; unexplained oligomenorrhea or amenorrhea; galactorrhea; unex-

plained hypertrophic osteoarthritis; new impaired glucose tolerance or diabetes as described above; and arterial hypertension [Grade A, Level 1].

Testing

IGF-I

The serum IGF-I test is simple to administer but, given the wide variations in results across laboratories and the lack of standardization of this test across Canada, the results may not always be solely reliable. Standardization of this test is needed, establishing normal age ranges with large cohorts. Until this is the case, the IGF-I test is useful as a screening tool and for diagnosis in conjunction with the 2-hour OGTT [Grade A, Level 1].

OGTT

All patients suspected of having acromegaly should undergo a 2-hour OGTT to monitor for growth hormone [Grade A, Level 1]. While IGF-I is simple to administer and can provide evidence of integrated growth hormone secretion, an OGTT is important because it can provide parametric measurement of growth hormone levels over time. It is a relatively simple test to administer, but it may be difficult to perform in outreach centres where there is less experience with this specialized procedure. The OGTT for growth hormone should be conducted for only 2 hours, because of the natural rise in growth hormone that normally occurs following the 2-hour interval.

The 2-hour OGTT test can be performed even in patients with overt diabetes,¹⁶ if it is conducted in a controlled environment in a medical facility; however, physicians may need to be sensitive to patient concerns in these circumstances and explain the reasons behind the test to allay any fears.

A normal GH response during an OGTT is to have at least one value less than 1 µg/L during the 2-hour test. An OGTT in which GH levels consistently remain above 1 µg/L throughout the test, in conjunction with the clinical picture and the results of IGF-I testing, is used to confirm a diagnosis of acromegaly.

Other testing

Other testing in conjunction with IGF-I and OGTT for diagnosis may include the following [Grade D, consensus]:

- Growth hormone-releasing hormone (GHRH) measurement for detection of an ectopic source of acromegaly, if this is suspected when no pituitary lesions are visualized despite elevated GH/IGF-I levels.

- Assessment of anterior pituitary function for all patients as a baseline: cortisol, prolactin, thyroid hormones (free thyroxine and thyroid-stimulating hormone), testosterone. Estrogen is not necessary if menses are intact.

- Determination of metabolic status (e.g., calcium, lipid profile, glucose tolerance), depending on the patient's clinical presentation.

Imaging

Magnetic resonance imaging (MRI) is the preferred imaging modality for diagnosis; computed tomography (CT) may be used if MRI is not available or if it cannot be performed (e.g., in a patient with a pacemaker) [Grade D, consensus].

Treatment

Surgical Therapy

Surgery should be considered as the first-line therapy in all patients with microadenomas and in those with non-invasive macroadenomas (Figure 1) [Grade A, Level 1]. In this group, there is a reasonable (60-80%) chance of remission with surgery alone.^{7,9,10} Even when complete resection is not possible, surgical decrease of tumour burden may relieve mass effects, such as vision loss, and possibly lead to more effective use of medical/radiation therapy.^{17,18} In cases of invasive macroadenomas, surgery alone is unlikely to normalize GH/IGF-I. Surgery is not recommended where the lesion cannot be identified with certainty. The only major contraindication for surgical treatment is poor medical condition (e.g., poor cardiac and pulmonary function); however, even in these cases patients' overall condition may be improved by medical pre-treatment, making them better future surgical candidates [Grade A, Level 1].^{7,19}

The goal of surgery for acromegaly is the normalization of GH and IGF-I levels, leading to alleviation of comorbidities and reduction in symptoms of mass effect (e.g., headache, vision problems).² The risks are the general risks of surgery and anesthesia, as well as possible postoperative pituitary insufficiency.²⁰ Perioperative glucocorticoids are required for patients who have abnormal preoperative morning cortisol levels (<450 nmol/L) or abnormal ACTH test.²⁰ To minimize risks, surgical resection should be performed in centres with experience in pituitary surgery, and by a multidisciplinary team with extensive experience in this area.²¹⁻²⁵

In general, surgical prognosis is better for smaller tumours.^{7-9,20,26-28} For this reason, improved screening and diagnosis are essential for ensuring that tumours

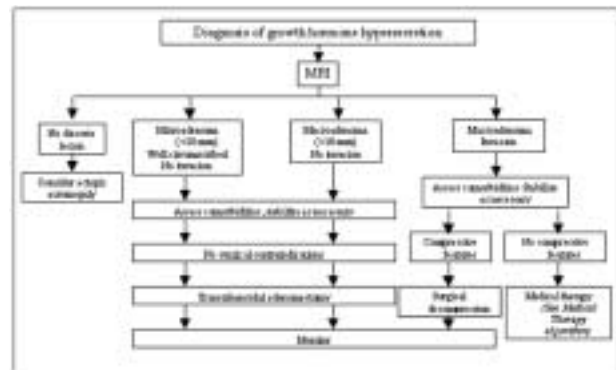


FIGURE 1 Treatment
MRI = magnetic resonance imaging

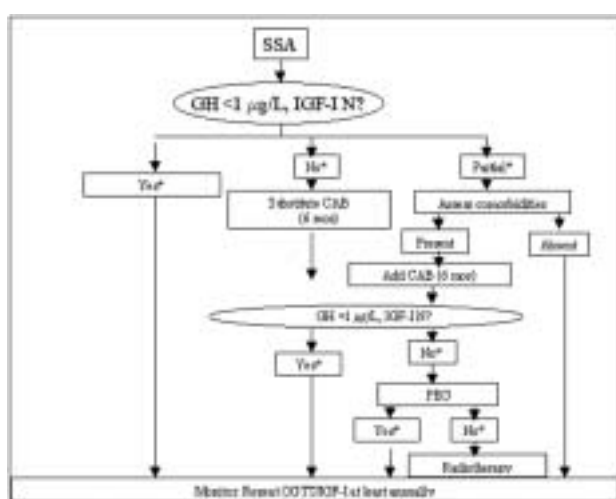


FIGURE 2 Medical therapy
CAB = cabergoline; GH = growth hormone; IGF-I = insulin-like growth factor-I; OGTT = oral glucose tolerance test; PEG = pegvisomant; SSA = somatostatin analogue
*Yes = >75% reduction in GH/IGF-I from baseline; No = <25% reduction in GH/IGF-I from baseline; Partial = 25-75% reduction in GH/IGF-I from baseline.

are identified and treated promptly, before reaching the stage of compressive or invasive macroadenomas.

Medical Therapy

Developments in medical therapy since the publication of the 2000 Canadian guidelines³ have included the increased availability of GH receptor antagonists

(pegvisomant) and better data to support their use, as well as further data to support the decreased use of dopamine agonists.

Candidates for primary medical therapy include those with macroadenomas unlikely to be cured by surgery, and those in poor medical condition, for whom surgery is unlikely to give good response or improved outcome [Grade D, consensus]. Secondary medical therapy should be considered for those in whom surgery has failed (persistently elevated IGF-I levels and non-suppression of GH $<1 \mu\text{g/L}$ during an OGTT) (Figure 2) [Grade A, Level 1].

Somatostatin analogues should be used as first-line medical therapy [Grade A, Level 1]. First-line or second-line therapy with a dopamine agonist (i.e., cabergoline) can be considered in co-prolactin-secreting adenomas and in patients with mildly to moderately elevated IGF-I levels (25-50% above upper limits of normal) [Grade C, Level 3]. A switch to a GH receptor antagonist (i.e., pegvisomant) can be considered in patients in whom other medical therapies have failed [Grade A, Level 1]; however, patients on pegvisomant therapy require special monitoring of the size of the pituitary lesion with MRI. If pegvisomant fails to normalize IGF-I levels or is contraindicated because of proximity of tumour to adjacent structures, radiotherapy should be considered (see Radiation Therapy, below).

Somatostatin analogues

The first-generation SSAs (e.g., octreotide) have a short half-life, requiring multiple daily subcutaneous injections. They have been shown to reduce GH and IGF-I in 50-70% of patients.²⁹⁻³¹ These agents result in maximal GH suppression in 2 hours, and the effect lasts approximately 6 hours.

Second-generation SSAs (octreotide LAR, lanreotide) have a longer duration of action (once-monthly intramuscular injection) and have clinical benefits similar to the short-acting formulations.³² Patient compliance can also be enhanced because of ease of administration and the more sustained effects of the long-acting formulation. The major barriers to their use are expense³³ and the fact that SSA treatment requires administration and monitoring by a health-care team.

Because of their rapid response and clearing, it may be helpful to use short-acting SSAs to assess potential response and any possible adverse effects before initiating treatment with long-acting SSAs.

SSAs have been shown to relieve acromegaly-related symptoms, such as headaches, sweating and arthral-

gias, in about 75% of patients. Some studies have shown reduction in tumour size, but in a smaller group of patients (30-50%),³⁴⁻³⁷ which may make SSAs a good option as secondary therapy following partial response to surgery.

The normalization of GH and IGF-I levels with SSA therapy has also been associated with an improvement in left-ventricular cardiac function,³⁸ since persistent high GH has been associated with higher blood pressure and impaired cardiac performance. Octreotide treatment has been associated with decreased prostate size and volume³⁹ and with favourable response in sleep apnea with the long-acting formulation.⁴⁰

Adverse events associated with all SSAs include abdominal cramps and diarrhea, but these tend to be temporary. SSAs have also been associated with increased incidence of gallbladder sludge and stones, but this is generally not of major clinical significance. Monitoring of blood glucose is also required to exclude the development of diabetes or glucose intolerance during SSA therapy.

SSAs should be considered as primary therapy for those who refuse surgery or who are poor surgical candidates. Some nonrandomized data have shown that SSAs are effective in the long term in reducing GH and IGF-I in patients who have not had pituitary surgery,²⁹ but the effects of SSAs on long-term acromegaly-related complications and mortality remain to be demonstrated.

Dopamine agonists

Dopamine agonists are now recommended less highly than they were in the previous Canadian guidelines, because they have been shown to be less effective than SSAs or GH-receptor antagonists. Drug-related adverse events associated with this class include GI discomfort and orthostatic hypotension.

Bromocriptine is generally not recommended for medical therapy of acromegaly.² Cabergoline, a more selective dopamine 2 receptor agonist, may have greater benefit than the other non-selective dopamine agonists in the treatment of this condition. It has been shown to lower IGF-I to $<300 \mu\text{g/L}$ in approximately 35% of patients^{41,42} (normal ranges: $<300 \mu\text{g/L}$ and $114-492 \mu\text{g/L}$). It may be considered as a first-line treatment in selected cases of co-secreting growth hormone and prolactin adenomas with mild to moderate elevations of IGF-I (25-50% above upper limits of normal). It may also be useful in combination with SSAs in patients in whom SSAs lead to only partial control of the disease.⁴³

GH receptor antagonists

This relatively new class of drugs blocks GH action directly by competing with natural GH for binding with the GH receptor, leading to reduced synthesis of IGF-I.

Pegvisomant is the only agent in this class currently available. Administered daily (10-20 mg sc), it has been shown to reduce and even normalize circulating IGF-I in >90% of patients.^{44,45} Nonetheless, its long-term effects on tumour growth and comorbidities remain to be established.

Pegvisomant is recommended for those in whom surgery, SSAs and dopaminergic agents have failed.

Radiation Therapy

Radiotherapy should be used following resection of as much of the adenoma as possible and should be viewed as a treatment of last resort, after all other options - surgical and medical - have been shown to be ineffective [Grade A, Level 1]. That is, if pegvisomant and all other medical therapies fail to normalize IGF-I levels following pituitary surgery, radiotherapy should be considered (stereotactic with gamma knife, if possible).

Conventional fractionated radiation may take 10-20 years to reach full effectiveness. In one study, it reduced serum GH to <5 µg/L in 77% of patients (15-year follow-up)⁴⁶; other studies have shown a reduction to <2.5 µg/L in 25% of patients (5-year follow-up)⁴⁷ and reduction of IGF-I to normal in 5% after 6.8 yr.⁴⁸

More recently, stereotactic radiotherapy has been used, including gamma knife radiosurgery. There is some suggestion that stereotactic radiotherapy may lead to earlier biochemical remission⁴⁹⁻⁵¹ than conventional radiotherapy. In one study, mean time to remission for gamma knife surgery was 1.4 yr, versus 7.1 yr for conventional radiotherapy⁴⁹; another study demonstrated a mean time to remission of 14 mo with gamma knife surgery.⁵⁰ It has been shown to lower IGF-I to normal in a number of studies.^{41,42,49} However, stereotactic methods should be used only when the distance between the tumour and the optic chiasm is >5 mm, because of the potential for vision damage.

The most serious potential complication of radiotherapy is loss of normal pituitary function. Conventional radiotherapy has the highest rates of post-therapeutic hypopituitarism, associated with up to 100% of patients,⁵² while gamma knife radiosurgery has been associated with lower rates of hypopituitarism (28%).⁴⁹ Other potential complications include radiation necrosis, loss of vision and development of a

TABLE 4. Symptoms and comorbidities to assess and monitor

Disease-related symptoms	<ul style="list-style-type: none"> • Headache • Fatigue • Hyperhidrosis
Metabolic comorbidities	<ul style="list-style-type: none"> • Diabetes mellitus • Dyslipidemia • Hypercalcemia
Cardiovascular comorbidities	<ul style="list-style-type: none"> • Cardiovascular disease • Hypertension • Left ventricular hypertrophy
Skeletal/dental comorbidities	<ul style="list-style-type: none"> • Arthritis • Carpal tunnel syndrome • Jaw malocclusion • Osteoporosis • Sleep apnea
Respiratory comorbidities	<ul style="list-style-type: none"> • Sleep apnea
Gastrointestinal comorbidities	<ul style="list-style-type: none"> • Colon polyps
Genitourinary comorbidities	<ul style="list-style-type: none"> • Prostate enlargement • Urinary stones
Other	<ul style="list-style-type: none"> • Hypopituitarism • Goitre/nodular thyroid

secondary malignant lesion. As well, because pituitary irradiation may impair fertility, the pros and cons of this therapeutic approach should be discussed in detail with young adults before beginning treatment.

Monitoring and Treating Comorbidities

Because of GH hypersecretion and its related metabolic effects, acromegaly is generally associated with a number of disease-related symptoms and comorbidities (Table 4).

Patients with acromegaly should be assessed for comorbidities upon diagnosis and receive regular follow-up to monitor for any change.

Monitoring

Patients with acromegaly should be assessed every 6 months for IGF/GH control, using the IGF-I and yearly using the 2-hour OGTT [Grade D, consensus]. Patients should also be monitored regularly for disease-related symptoms (to assess the success of treatment), and for evidence of comorbidities, as required [Grade D, consensus].

Depending on the patient's existing comorbidities and risk factors, monitoring should also include standard

testing for metabolic abnormalities (e.g., lipid testing), cardiovascular risk factors (e.g., echocardiography), musculoskeletal abnormalities (e.g., bone mineral density scanning), respiratory problems (e.g., sleep apnea assessment), gastrointestinal disorders (e.g., colonoscopy) and genitourinary abnormalities (e.g., prostate examinations, kidney scans) [Grade D, consensus].

Monitoring for hypopituitarism after surgery and/or radiotherapy should include metabolic monitoring and imaging (MRI, not CT), especially for patients taking pegvisomant [Grade D, consensus].

Treating Comorbidities

Monitoring and treating the comorbidities associated with acromegaly are essential for improving the quality of life of patients and probably reducing the increased mortality associated with this disease.² This means modification of risk factors; early diagnosis; and careful management of comorbidities.

Cardiovascular disease

Patients with acromegaly and cardiovascular disease or cardiovascular risk factors will benefit from normalization of GH and IGF-I. Research has shown that left-ventricular size and function may improve when GH levels are returned to normal.⁵³ Diabetes, glucose intolerance and hypertension may also be improved with normalization of IGF-I levels.¹⁹

Patients with left-ventricular hypertrophy, impaired cardiac systolic and diastolic function, arrhythmias, conduction abnormalities, valvular heart disease and ischemic heart disease should be treated using standard therapies [Grade A, level 1].^{2,54} Standard dietary methods may be used to manage diabetes mellitus, hypertension and hyperlipidemia [Grade A, level 1]. Treatment targets for cardiovascular comorbidities may be found in the most recent relevant Canadian guidelines.^{1,55-57}

Skeletal/dental comorbidities

Skeletal and/or dental comorbidities benefit most from early diagnosis and normalization of GH levels, since the bone enlargement associated with acromegaly, unlike the soft-tissue overgrowth, is not easily reversed with normalization of hormone levels [Grade D, consensus].⁵⁸ Corrective surgery may be required to treat bone enlargement, but this should not be scheduled until after normalization/stabilization of hormone levels.

Hypercalcemia/hypercalciuria may occur with high levels of GH, and diminish with successful treatment.

Arthropathy and carpal tunnel syndrome may be due in part to soft-tissue overgrowth and may be relieved somewhat when excess GH secretion is normalized, but degenerative arthritis is irreversible and should be treated appropriately.

Patients with acromegaly should be screened for osteoporosis. If osteoporosis is present and is not corrected by hormone stabilization, consider antiresorptive therapy [Grade D, consensus].⁵⁹

Respiratory disorders

Because patients with acromegaly have a high prevalence of obstructive pulmonary disease, putting them at a higher-than-normal risk for pulmonary infection and its associated mortality,⁴⁸ they should be vaccinated for influenza and pneumococcal pneumonia^{60,61} and be recommended for a smoking cessation program, if necessary [Grade D, consensus].

Normalization of GH hypersecretion may improve symptoms of sleep apnea; nevertheless, sleep studies should be performed to determine the source of the sleep apnea (central or obstructive) and determine a course of treatment [Grade D, consensus].⁶²

Gastrointestinal disorders

Acromegaly is associated with an increased risk of precancer colon polyps,^{63,64} but any demonstrated increased prevalence of colon cancer remains controversial.⁶⁵ However, there is an increased risk of death in those who do develop colon cancer.⁶⁶ Screening and early detection may improve survival, and monitoring for and removal of precancerous polyps will prevent development into cancer.⁶⁷ Colonoscopies are recommended particularly in newly diagnosed patients, those over the age of 50, those with persistently active disease or those with previously identified polyps [Grade A, Level 1].⁶⁶

Genitourinary disorders

Patients with acromegaly are at increased risk for prostate enlargement, and for urinary stones. Standard monitoring and treatment should be applied [Grade D, consensus].

Hypopituitarism

The risk of developing hypopituitarism depends on how acromegaly is treated. Hypopituitarism resulting from the compressive effects of a tumour may actually be relieved by surgical decompression, but new hypopituitarism may occur as a result of surgery or radiotherapy. Pituitary function should be assessed in

the immediate post-treatment period. Early postoperative assessment depends on daily clinical assessment of the patient. A morning plasma cortisol >450 nM reflects normal hypothalamic-pituitary-adrenal axis function, and levels less than 100 nM are suggestive of ACTH deficiency. Those with values <450 nM should be retested using the insulin tolerance test as early as 7-10 days after surgery or, if more convenient, 4-6 weeks postoperatively [Grade D, consensus].²⁰ Adrenal, thyroid and gonadal axes should be assessed 6-12 weeks post-surgery [Grade D, consensus]. Monitoring of pituitary function after radiotherapy should be lifelong, as hypopituitarism may develop decades following treatment [Grade D, consensus].²

Conclusion

Acromegaly is a chronic condition associated with increased morbidity and mortality if left unchecked. Diagnosis should include clinical and biochemical findings, but is hinged on establishing GH hypersecretion with IGF-I and OGTT testing. Treatment has traditionally included surgical resection or debulking, along with adjunctive medical therapy (primarily somatostatin analogues), if necessary, to normalize GH levels. The option of primary medical therapy in managing this condition has recently emerged and can be justified for non-surgical candidates or for those in whom surgery is not expected to be curative. Overall, improved screening practices and superior epidemiological data are required, since timely diagnosis and appropriate treatment are crucial for reducing the potentially debilitating effects of this chronic, progressive disease. The current evidence also supports the need for long-term follow-up of disease activity and comorbidities in diagnosed patients.

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RCPSC/CSCI Awards

1. Henry Friesen Award

Recipient - Dr. Katherine Siminovitch



Dr. Katherine Anne Siminovitch

Dr. Siminovitch is a Professor of Medicine at the University of Toronto and a Senior Scientist and Director of the Genomic Medicine Division at the Mount Sinai Hospital Samuel Lunenfeld Research Institute. She also serves as the Director of a Gene Profiling Facility and Adult Clinical Genetics service at the University Health Network and is the Director of the Molecular Therapeutics Program at the University of Toronto McLaughlin Molecular Medicine Centre. She trained in Internal Medicine and Rheumatology at the University of Toronto, and received post-doc-

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INFLAMMATORY BOWEL DISEASE IN THE ERA OF GENOMIC MEDICINE

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INTRODUCTION

Knowledge emanating from the Human Genome Project has provided an unprecedented opportunity to advance understanding and management of disease. Together with information on the full human genome sequence, the high throughput analytic technologies, computational tools and rich polymorphic marker resource derived in conjunction with the genome project have rendered feasible the genetic dissection of common disease and definition of the molecular pathways coupling specific gene variants to pathology and disease. Such knowledge has many potential clinical benefits, enabling, for example, the definition of mol-

ecular biomarkers for improved diagnosis and risk prediction, discovery of new therapeutic targets, and ultimately, “individualized” medical care predicated upon genetic stratification of disease. By deploying the wealth of genetic data and rapidly evolving genomic technologies towards discovery of the complement of gene variants and genetic-environmental interactions underlying individual common diseases, it should be possible to reap these benefits and translate the promise of the Genome Project into tangible health-care benefit.

Molecular genetic technologies have been very successfully applied to the identification of genes involved in Mendelian-inherited “single-gene” diseases. A much greater challenge, however, is posed by the search for genes involved in diabetes, multiple sclerosis, atherosclerotic cardiovascular disease, and the many other common, genetically-complex diseases which collectively account for most of the healthcare burden in developed countries. Such conditions are multifactorial in etiology and the mode of inheritance and penetrance of the disease-causal alleles are usually complex and poorly defined. Despite these confounding factors, the successful use of gene mapping strategies over the past few years to identify gene variants for several complex phenotypes indicates that the genes for common diseases are amenable to genetic dissection.^{1,2} This conclusion is particularly well-supported by recent achievements in the mapping and characterization of inflammatory bowel disease (IBD) susceptibility genes. This review outlines some of the major successes in identifying IBD susceptibility loci and alleles with a specific emphasis on the possible contributions of variants in the CARD15 and SLC22A4/SLC22A5 genes to risk for IBD and pathophysiology of disease.

Inflammatory Bowel Disease

The inflammatory bowel diseases, Crohn’s disease (CD) and ulcerative colitis (UC), are characterized by chronic intestinal inflammation which leads to damage and destruction of the intestinal mucosa. Prevalence of IBD is 200-300/100,000 in industrialized countries, but epidemiologic data reveal a widespread steady increase in the incidence of CD.³ Like most chronic diseases, the etiology of IBD is thought to reflect a complex interplay between multiple genetic and environmental factors. Epidemiologic data, for example, indicate concordance for CD in European twin pairs to be about 36% in monozygotic versus 5% in dizygotic twins, risk for IBD to be 10-20 fold higher in first degree relatives of affected individuals than in the general population, and IBD prevalence to be 2-8 fold higher in Ashkenazi Jewish than most other populations.⁴⁻⁷ Many environmental factors such as smoking, diet, oral contraceptives and microbial pathogens, have also been implicated in IBD etiology.⁸⁻¹¹ However, validation of such factors as triggers for IBD has been very difficult and will likely require unraveling the genetic basis for IBD and subsequent prospective studies of high risk cohorts.

Gene Mapping in IBD

Genome wide screens of IBD families have identified at least 9 putative IBD susceptibility loci (Table 1). These include, for example, three loci on chromosomes 16q12 (IBD1), 14q11-12 (IBD4) and 5q31 (IBD5), respectively, which show linkage exclusively to CD, the IBD2 locus on chromosome 12q14 showing linkage primarily to UC, and several loci such as IBD3 on 6p21 and IBD6 on 19p13 showing significant linkage to IBD in both individual genome-wide screens of IBD families and in a meta-analysis consolidating linkage data from ten such screens.¹²⁻²⁴ While only a few of these loci, such as IBD1 and IBD3, have

TABLE 1: Location of major IBD susceptibility loci identified by genome-wide screens.

<i>Susceptibility Locus</i>	<i>Chromosomal Region</i>	<i>Gene Implicated</i>	<i>Linked and/or Associated Disease</i>
IBD1	16q13	CARD15	CD
2	12q14	Unknown	Primarily UC
3	6p21	Unknown	IBD
4	14q11-q12	Unknown	CD
5	5q31	SLC22A4/A5	Primarily CD
6	19p13	Unknown	IBD
7	1p36	Unknown	IBD
8	16p12	Unknown	CD
9	3p26	Unknown	IBD

been widely-replicated in independent genome-wide surveys, evidence supporting involvement of less-frequently replicated loci (such as IBD2, IBD4, and IBD5) has been derived by either larger scale replication studies incorporating IBD families from many centres^{20,22} or from genetic association studies.²⁵⁻²⁹ Together these data have served to confirm the existence of multiple IBD susceptibility loci, to show that at least some susceptibility loci differ between CD and UC, may differ in different populations, and act epistatically to predispose to IBD^{14,19,23} and, importantly, to enable identification of several IBD susceptibility genes.

CD and the CARD15 Gene

The CD susceptibility gene at the IBD1 locus was discovered in 2001 by two groups who respectively used positional cloning and candidate gene strategies to identify CARD15 (aka NOD2) as the gene within this region involved in IBD.^{31,32} As shown in Figure 1, the gene encodes a cytosolic protein including two N-terminal caspase recruitment domains (CARDs), a central nucleotide binding domain (NBD) and a C-terminal region comprised of leucine rich repeats (LRR). Studies of CARD15 genotypes have revealed that three major CARD15 mutations (Arg702Trp, Gly908Arg and Leu1007finsC) mapping in and around the LRR account for about 80% of the CARD15 variation associated with CD.^{33,34} Among Caucasian whites, at least one of these three variants occurs in 30-40% of CD patients and in 15-20% of healthy controls and increases risk for CD by 1.5-4 fold when carried as a single allele and by 15-40 fold when carried as two alleles.³²⁻³⁸ However, CARD15 variants are very rare and are not IBD risk alleles in African and Asian populations.^{39,40} The relationship of CARD15 with many other facets of CD phenotype and demographics has been extensively studied and the results of these studies indicate the presence of CARD15 variants in CD patients to be consistently associated with the presence of ileal disease,³²⁻³⁸ but not consistently (i.e. across all studies) associated with any other disease characteristic such as fistulizing and fibrostenotic behaviour, age at onset, or rate of progression.^{41,42} Together, the available data indicate an important role for CARD15 mutation in risk for CD, but also reveal CARD15 variants to be neither necessary nor sufficient for expression of CD and therefore, at least when considered in isolation, to have a limited role in disease prediction and management.

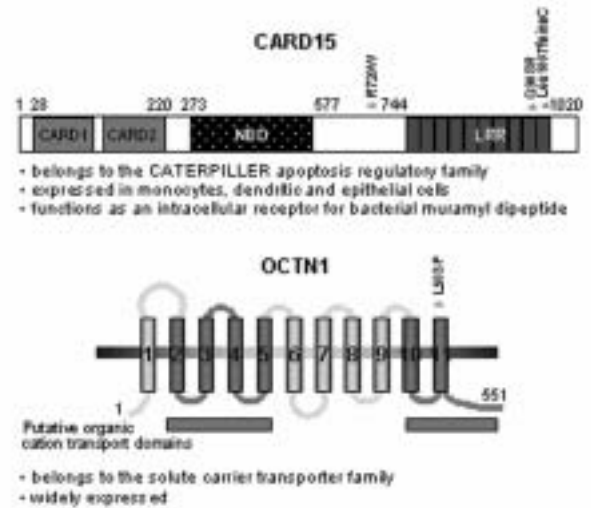


FIGURE 1: Diagram showing CARD15 and OCTN1 domain structure. The boundaries of the CARD15 caspase recruitment domains (CARDs), nucleotide binding domains (NBD) and leucine rich region (LRR) are indicated numerically and the positions of the three major CD-associated variants are shown by asterisk. OCTN1 transmembrane topology and putative cation transport regions and the CD-associated L503F variant are also indicated.

IBD5 and the SLC22A4/SLC22A5 Risk Alleles

A locus at chromosome 5q31 showing significant linkage to CD was initially identified by our group in a genome-wide screen of 158 Toronto-based sib-pair families.²¹ Linked most strongly with early onset CD in this screen, the IBD5 locus was also detected in several other independent genome-wide scans^{14,17} and was later replicated and refined to a 1 megabase interval by evaluation of 256 parents/affected offspring trios for CD association with additional microsatellite markers spanning the linkage region.²⁵ Through extensive resequencing across this segment, about 650 single nucleotide polymorphisms (SNPs) were identified, 300 of which were used to re-genotype the CD trio families and to thereby further refine the interval via association analysis. Results of these analyses revealed extensive linkage disequilibrium (LD) across the region and enabled the IBD5 interval to be refined to a 250 kb block defined by a risk haplotype comprised of 11 SNP alleles in tight LD with one another. Association of IBD with this IBD5 “risk haplotype” has since been replicated in numerous independent studies, the majority of which reveal the

association to be detectable in CD, but not UC patients.²⁶⁻²⁹ Relevance of this locus to disease behaviour is, however, unclear, data from some, but not all studies revealing positive association of the IBD5 haplotype with early onset and perianal disease in CD patients and, very recently, with poor response to infliximab therapy.^{26,27,29} Moreover, as for the CARD15 variants, IBD5 relevance to IBD susceptibility appears variable in different populations, this association not apparent in the Japanese population and involving UC as well as CD in some populations.^{28,30}

Following refinement of the IBD5 interval, our group undertook extensive sequencing across all of the genes and predicted gene sequences within this interval and thereby identified a C→T transition (C1672T) in the solute carrier family SLC22A4 gene and a G→C transition (G-207C) in the SLC22A5 gene promoter region, which together comprise a two-allele risk haplotype (SLC22A-TC) strongly associated with CD.⁴³ SLC22A4 and SLC22A5 are physically contiguous genes that respectively encode OCTN1 and OCTN2, two functionally-related integral membrane proteins that mediate transport of carnitine and a wide range of cationic compounds (Fig. 1).⁴⁴ Evaluation of Canadian IBD cases and controls for these variants has revealed frequency of the SLC22A-TC risk haplotype to be about 54% in CD cases and 42% in healthy controls, a single copy of the haplotype increasing risk for disease by 2.5 fold and two copies increasing risk by 4 fold.⁴⁵ Within this population, this haplotype and risk alleles occur without the IBD5 risk haplotype significantly more frequently in CD patients and controls, and appear to interact with CARD15 variants in predisposing to CD and to ileal disease among CD patients (Fig. 2). As with the IBD risk haplotype, the SLC22A-TC haplotype was not associated with CD in the Japanese population, although weak association of a different, intronic SLC22A4 variant with CD has been detected in this population.⁴⁶ By contrast, association of the SLC22A-TC haplotype with CD has recently been replicated in a German IBD cohort, the data revealing this haplotype to be associated with colonic, non-fistulizing and early onset CD, to interact with CARD15 alleles in conferring risk for CD, and to show no association with UC.⁴⁷ Together these data suggest that effects of the IBD5 locus and the SLC22A-TC alleles within this locus on both IBD susceptibility and behaviour differs among ethnic groups and require further dissection by evaluation of larger cohorts and the biological sequelae of risk variant carriage.

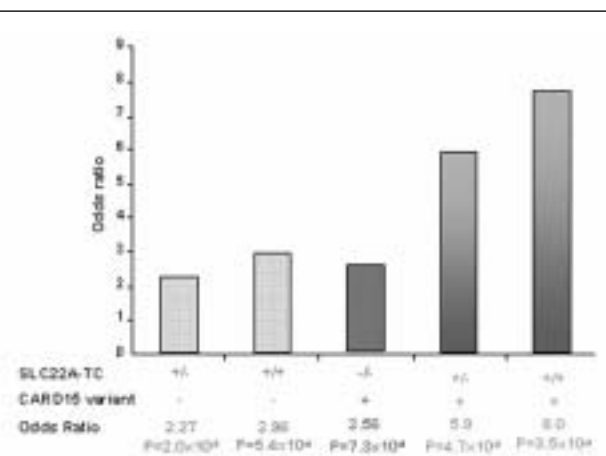


FIGURE 2: The SLC22A-TC risk haplotype interacts with CARD15 variants to confer risk for CD. Bar graphs correspond to the odds ratios and p-values for association of CD with heterozygosity (+/-) or homozygosity (+/+) for the SLC22A-TC risk haplotype in the absence (-) or presence (+) of a CARD15 variant and for CD association with CARD15 variant carriage in the absence of the SLC22A-TC haplotype in a Canadian CD cohort.

CARD15 Functions in Relation to CD

Following the identification of CARD15 as a susceptibility gene for CD, the mechanisms whereby CARD15 variants predispose to disease have been extensively investigated. CARD15 is now known to be expressed in monocytes, macrophages and dendritic cells as well as small intestinal Paneth and other epithelial cells.⁴⁸⁻⁵⁰ The structural organization of CARD15 (Fig. 1) is similar to that of so-called pattern-recognition receptors, proteins which recognize bacterial motifs (pathogen-associated molecular patterns) and thereby link pathogens to the induction of protective immune responses.⁵¹ The CARD15 LRR has been shown to mediate CARD15 interaction with muramyl dipeptide (MDP), a breakdown product of bacterial cell wall peptidoglycan which is released from phagocytes and then actively taken up by intestinal Paneth cells via the hpept1 intestinal transporter.^{52,53} Following interaction with MDP, CARD15 oligomerizes, binds via its CARDs to the RICK serine/threonine kinase and thereby evokes RICK binding to the IKK complex and induction of NF- κ B activation (Fig. 3).^{54,55} MDP binding also triggers CARD15-mediated induction of defensins, a class of antimicrobial proteins produced by Paneth cells and implicated in inactivation/killing of

both commensal and pathogenic bacteria.⁵⁶ While the functional properties of CARD15 are highly consistent with its involvement in IBD, definition of the pathways linking CARD15 dysfunction to CD has not been straightforward. For example, CARD15 deficiency was shown in one study to be associated with enhanced Toll-like receptor-2 (TLR2) mediated activation of NF- κ B, suggesting that CD-associated mutations in CARD15 impede its normal downregulation of TLR2 signaling so as to augment TLR2/NF- κ B-induced proinflammatory cytokine production.⁵⁷ However, these data have not been widely replicated and, conversely, a positive regulatory role for CARD15 in relation to TLR2 signaling has also been reported.⁵⁸ Moreover, MDP-induced NF- κ B activation was shown in another study to be markedly reduced in CARD15-deficient bone marrow dendritic cells,⁵⁹ but was found to be elevated in macrophages from mice expressing a CD-associated mutant CARD15 rather than the wild-type protein.⁶⁰ However, while not fully characterized as of yet, CARD15 capacity to connect microbial organisms to immune response is highly consistent with its involvement in CD and the dissection of the pathways linking CARD15 variants to this disease will almost certainly impact significantly on the understanding of IBD etiology.

OCTN1/OCTN2 Functions in Relation to CD

The OCTN1 and OCTN2 proteins encoded by the SLC22A4 and SLC22A5 genes are highly conserved and widely expressed bidirectional transporters which carry carnitine and organic cations such as TEA across the plasma membrane.⁴⁴ The two variants in these genes shown to be associated with CD respectively induce a leucine to phenylalanine substitution at position 503 within an OCTN1 transmembrane domain and a single base substitution within a heat shock element in the OCTN2 promoter region (G-207C). Both of these changes have functional consequences, the OCTN1 L503F substitution altering OCTN1 carnitine and TEA transporter activities and the -207G>C variant disrupting heat-shock inducible transcriptional activation of the OCTN2 promoter.⁴⁵ These defects may therefore influence IBD susceptibility via several mechanisms. Carnitine, for example, is an essential mediator of cellular fatty oxidation and impaired fatty oxidation has been linked to the induction of rodent colitis.^{61,62} These transporters also accept a broad range of substrates, including many xenobiotics, which may adversely alter intestinal epithelial cell integrity/function if abundantly accumulated in these cells. Importantly,

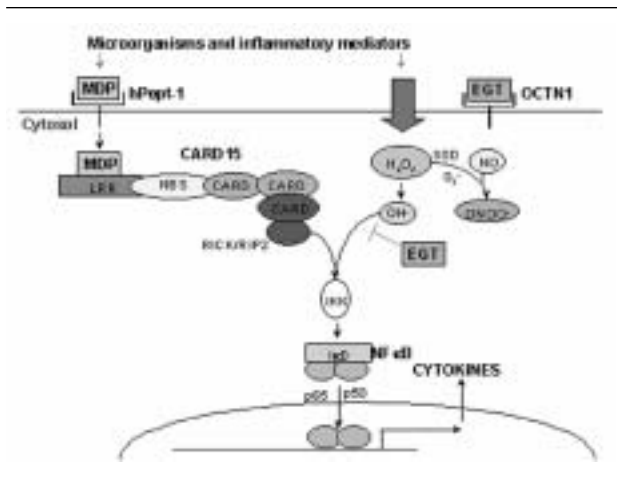


FIGURE 3: CARD15 and OCTN1 activities may coordinately modulate NF- κ B activation. Bacterial muramyl dipeptide (MDP) released from phagocytic cells and transported into intestinal epithelial cells by the hPept1 brush border transporter interacts with the CARD15 LRR so as to induce CARD15 oligomerization and RICK activation. RICK then binds the IKK complex and thereby triggers NF- κ B activation. Intracellular transport of ergothioneine (EGT) via the OCTN1 transporter enables targeting of EGT antioxidant activity at reactive oxygen species induced by oxidative stress/inflammatory mediators and thereby uncouples such species from NF- κ B activation. The L503F OCTN1 variant may impede EGT transport and thereby allow for enhanced induction of NF- κ B activation via cytokine or bacterial-driven signaling pathways. Thus variants in both CARD15 and OCTN activities may coordinately modulate NF- κ B activation.

recent studies of OCTN1 substrate specificity have revealed its physiological substrates to also include ergothioneine (EGT), a naturally occurring antioxidant produced in bacteria and fungi and absorbed in humans primarily from dietary sources.⁶³ The biological properties of EGT are not well characterized, but this compound has significant radio-protective and anti-inflammatory effects and functions as a scavenger of oxygen, hydroxyl and peroxy radicals and an inhibitor of peroxide formation in some systems.⁶⁴ Importantly, EGT has also been shown to inhibit the induction of NF- κ B engendered by either oxidative stress or pro-inflammatory cytokines such as TNF- α .⁶⁵ Thus NF- κ B may represent an effector common to both CARD15 and OCTN1-regulated signaling pathways, CARD15 promoting NF- κ B activation through MDP-evoked CARD15-RICK interaction, and OCTN1 potentially serving to inhibit NF- κ B activation by transport of EGT intracellularly with consequent

downregulation of NF- κ B activity (Fig. 3). Although purely speculative at the present time, the possibility of CARD15-OCTN1 cross-talk at the level of NF- κ B provides an attractive explanation for genetic data suggesting joint effects of CARD15 variants and the SLC22A-TC risk haplotype on IBD susceptibility.

CONCLUDING REMARKS

In addition to CARD15 and SLC22A4/A5, many other genes have been extensively explored as candidate susceptibility genes for IBD. These include, for example, the HLA genes which map within an IBD linkage interval (6p21) and for which multiple specific alleles associated with CD and UC have been identified, albeit not consistently replicated.^{66,67} Similarly, the DLG5 (*Drosophila Discs Large Homologue*) gene mapping to a linkage interval on 10q22-23, has been shown in a few studies to carry variants associated with IBD, although again these findings have not been widely replicated.⁶⁸ Many other genes have also been shown to carry variants associated with IBD and while most of these findings require additional replication and biologic validation, the cumulative genetic linkage and association data highlight the genetic heterogeneity of IBD and suggest that incorporation of genetic data into clinical practice will require definition of the full complement of alleles conferring risk to IBD. This caveat notwithstanding, the discovery in recent years of multiple IBD linkage intervals reproducibly detected in independent populations and the successful definition of gene variants shown widely to act as susceptibility alleles for CD, attests to remarkable progress in the field of IBD genetics and to the potential for disease gene mapping strategies to provide the genetic knowledge required for comprehensive understanding and ultimately more efficacious clinical management of IBD.

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2. Distinguished Scientist Award

Recipient - Dr. Leo P Renaud



Leo P. Renaud

Leo Renaud graduated in Medicine from the University of Ottawa in 1965. After clinical and basic research training at McGill University that included a PhD in Neurophysiology, he joined the Dept of Neurology & Neurosurgery at the Montreal General Hospital in 1973. As an MRC Scholar and a founding member of the Centre for Research in Neuroscience, he balanced his time between clinical neurophysiology (EEG and EMG clinics) and the development of an internationally recognized basic neuroscience team focused on synaptic transmission in hypothalamic neurosecretory neurons. A strong supporter of research at many levels, he has seen service on many intra- and extra-university committees, including President of the Canadian Physiological Society, and the Canadian Association for Neuroscience. In 1990, he accepted the offer to assist in the development of clinical and basic neuroscience at the University of Ottawa, initially at the Civic Hospital campus, now more broadly within the merged Ottawa Hospital. While sustaining a basic neuroscience focus, he currently supports research as Director of Research in the Department of Medicine, and as an Associate Director of the Ottawa Health Research Institute. In 1995 he received a prestigious 5 yr MRC Distinguished Scientist award, and in 2000 was appointed as a Fellow in the Royal Society of Canada.

Vasopressin: a neuropeptide with multiple roles, from antidiuresis to neural regulation of monogamy

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The discovery in the late 1800s that extracts of posterior pituitary could induce a rise in arterial blood pressure was one of the key forerunners of more than a century of progress in neuroendocrinology that has seen the neurohypophysial peptides oxytocin and vasopressin emerge as models for molecules with dual roles: hormones in the circulation, and neurotransmitters within the central nervous system. Du Vigneaud (1955) and Acher (Acher and Fromageot, 1955) independently recognized their different amino acid composition, a

discovery that was critical to opening avenues for their full and differential functional characterization. This was followed by the development of selective antibodies for immunocytochemistry and the revelation that vasopressin and oxytocin were not just confined within the hypothalamo-neurohypophysial system (HNS) but were synthesized by selective neurons whose axons were differentially distributed throughout the brain and spinal cord (c.f. Buijs 1978). As a neuroscientist, my interest has focused on the neurophysiology of central neurons that control pituitary and autonomic functions. Both historically and practically, the magnocellular vasopressin-synthesizing neurons that constitute the HNS, the “classical neurosecretory neurons”, present an interesting and accessible model system to explore this topic. This paper will initially review aspects of our contribution to understanding their neurophysiology and pharmacology, highlight ongoing investigations into the transmitter-like actions of vasopressin, and how vasopressin receptors regulate central neuronal excitability. To present this peptide in its broader functional context, this will be followed by notes on the cellular biology of co-peptide synthesis and distribution in CNS neurons, a role for vasopressin as a stress-activated hormone, and roles attributed to vasopressin and vasopressin receptors in higher functions, notably social recognition, and circadian rhythmicity.

Profiling the vasopressin-synthesizing magnocellular neurosecretory neuron.

Studies on the antidiuretic and pressor capabilities of posterior pituitary extracts in the early 1900s became more neuronal in nature by the 1950s after Verney and colleagues reported that the anterior hypothalamus, in particular the area of the supraoptic nucleus (SON), was critical for sensing and responding to an osmotic stimulus. Many integrative, cellular and molecular studies have since confirmed that vasopressin-synthesizing neurons in the SON and magnocellular paraventricular nucleus (mPVN) constitute a target for converging projections that promote the release of vasopressin (and oxytocin) from posterior pituitary axon terminals into the plasma i.e. neurosecretion. A variety of challenges to homeostasis (notably hydromineral, cardiovascular, immune) trigger a rise in circulating vasopressin. Facilitated by anatomical tracers, by the detection of early active gene products responding to novel stimuli (e.g. see Dampney et al., 2003) and by in-vivo electrophysiology, it has been possible to map out the neuronal circuitry responsible for transmitting the sensory components to the vasopressin-secreting neurons.

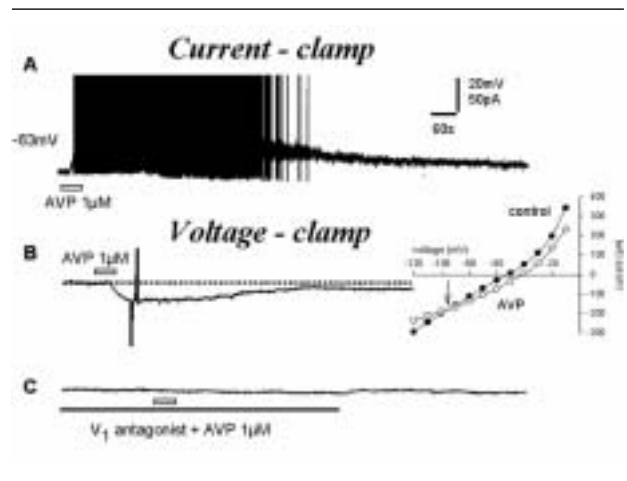


FIG 1. Schematic summary to indicate possible consequences of vasopressin release into the blood (neuroendocrine arrow), cerebrospinal fluid (circadian arrow) and at central synapses (remaining arrows), depicting the types of vasopressin receptors likely to mediate the peptide's actions. See text for details.

Contributions from electrophysiology. Our earlier research addressed the electrophysiology of vasopressin-ergic neurons. In-vivo extracellular recordings in the anesthetized rat model presented two opportunities that are unique to the hypothalamus: the ability to identify recordings from magnocellular neurosecretory neurons based on their antidromic activation; the ability to identify the vasopressin-synthesizing neurons selectively by their electrical signature (phasic firing, and transient cessation inhibition of firing in response to an abrupt drug-induced rise in blood pressure). As reviewed (see Renaud and Bourque, 1991), this approach successfully allowed us to characterize the aspects of their cellular electrophysiology and neuropharmacology. In vivo studies are important for appreciating and analyzing the “integrative” aspects of central autonomic regulation, and our research also served to clarify some controversial issues, notably the functional nature of ascending medullary-hypothalamic catecholaminergic pathways. Whereas lesion-based investigations suggested that the ascending noradrenergic system suppressed vasopressin secretion, our analyses incorporating data from both in-vivo and in-vitro preparations convincingly demonstrated that noradrenaline excited vasopressin-secreting neurons by its action at postsynaptic ± 1 adrenoceptors, mediated via suppression of a potassium conductance (reviewed in Renaud and Bourque, 1991).

With the introduction of intracellular and patch clamp recording techniques in in-vitro preparations,

much more detailed analysis of the synaptic activity engaging magnocellular neurons has been possible (e.g. Ludwig and Pittman, 2003; Olié et al., 2004). Witness the case of noradrenaline where presynaptic ± 1 adrenoceptors have been reported to increase excitatory glutamatergic inputs via a PKC-mediated suppression of group III metabotropic glutamate receptors, a long lasting action that may 'prime' synapses for subsequent actions of noradrenaline (Gordon and Bains, 2003). Noradrenaline may also promote glial cells to release ATP which can act via postsynaptic $P2X_7$ receptors on magnocellular neurons to increase the amplitude of miniature excitatory postsynaptic currents (Gordon et al., 2005). Additionally, these in-vitro approaches have allowed for a more detailed account of the intrinsic membrane properties and conductances that are unique to magnocellular neurons, and serve to distinguish vasopressin- from oxytocin-synthesizing cells (e.g. Armstrong and Stern, 1998).

Vasopressin neurons as osmoreceptors. A particularly relevant contribution to osmotic regulation of vasopressin secretion derives from in-vitro electrophysiological studies. Neurons in both the organum vasculosum lamina terminalis (located at the ventral edge of the anterior wall of the third cerebral ventricle) and the vasopressin-synthesizing magnocellular neurons themselves are intrinsically osmosensitive (Bourque et al., 2002; Voisin and Bourque, 2002). This osmosensitivity is attributed to intrinsic mechanosensitive stretch-inactivated cation channels that sense changes in cell volume ensuing from a deviation from resting plasma osmolality (~ 290 mosmol/kg in humans). A rising extracellular osmolality causes shrinkage and cytoskeletal changes promoting an increase in a membrane cationic conductance, membrane depolarization and discharge of action potentials. In vivo, these action potentials would then propagate into axon terminals in the neurohypophysis where a stimulus-secretion coupling mechanism would promote exocytosis of the stored peptide from neurosecretory granules. Another property of vasopressin neurons that facilitates hormone release in the neurohypophysis in response to a hyperosmotic stimulus is both an increase in their firing frequency (up to ~ 20 Hz) and a transition in firing pattern from slow-irregular to phasic bursting modes, both serving to enhance stimulus-secretion coupling and vesicle exocytosis from their axon terminals.

Electrophysiology of vasopressin receptors. Two classes of G protein-linked membrane bound receptors mediate responses to vasopressin. V_1 receptors consist of

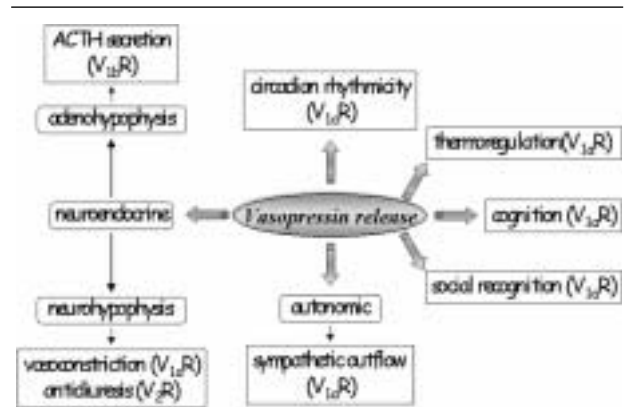


FIG 2. Electrophysiology of vasopressin V_1 receptors in neonatal rat spinal anterior horn neurons. Traces of data obtained with patch clamp recording techniques. A: Trace obtained in current clamp mode demonstrates that bath application of arginine vasopressin (AVP, open bar) results in a slowly rising membrane depolarization that triggers a burst of action potentials; not the prolonged duration of the effect after the peptide application. B: A subsequent trace obtained in voltage clamp mode demonstrates inward current associated with the vasopressin application. On the right, voltage-current plots before (solid dots) and during (open dots) the response to vasopressin display a reduction in conductance, and their crossing around -95 mV (arrow) suggests that the depolarization arises due to closure of a membrane potassium conductance:

two subtypes: V_{1a} in vasculature smooth muscle, liver and most areas of brain, and V_{1b} in the anterior pituitary; their activation results in the hydrolysis of phosphatidylinositol and an increase in cytosolic calcium. V_2 receptors, which act via stimulation of adenylate cyclase are most abundant in renal tubules where they promote free water re-absorption through stimulating translocation of aquaporin 2 water channels to the apical membrane in collecting duct cells.

The presence of vasopressin fibers in spinal cord (Buijs, 1978) together with evidence that exogenous vasopressin could enhance neuronal excitability in a variety of brain regions prompted us to explore the effects of exogenous vasopressin on neonatal neurons in the lateral and ventral spinal cord. Using patch clamp recording techniques in brain slices, we observed that a majority of neurons, including spinal preganglionic neurons, responded to vasopressin in two ways: a) a prolonged V_1 postsynaptic receptor-mediated membrane depolarization and inward current that involved two conductances, a reduction in

one or more barium-sensitive potassium conductances and an increase in a non-selective cationic conductance; b) a *presynaptic action that had two components: one was activity-dependent*, due to vasopressin's excitatory effects on inhibitory and excitatory neurons that synapse onto the recorded neurons; a second action that was activity-independent and due to direct activation of presynaptic V_1 receptors on the excitatory and inhibitory terminals synapsing on the recorded cells (see Oz et al., 2001).

Surprise at this potent and widespread response to vasopressin in an area that lacks a density of vasopressinergic input prompted us to evaluate the effects of other neuropeptides on neonatal spinal cord neurons. Indeed we have now seen basically similar responses with thyrotropin-releasing hormone (Kolaj et al., 1997) and angiotensin (Oz et al., 2005). This leads one to suggest that these neuropeptide receptors, in particular vasopressin V_1 receptors, may have more than a neurotransmitter or neuromodulatory function, perhaps serving a neurotrophic role in the developing spinal cord (Iwasaki et al., 1991), perhaps being up- or down-regulated, or possibly re-expressed, under certain conditions such as neuronal injury (see Tribollet et al., 1994).

Vasopressin synthesis, co-expression, differential peptide processing.

First described in the fish CNS in 1928 (Scharrer, 1987), the HNS has been a rich source of information and model system to study peptide biosynthesis and processing. Within minutes of a physiological stimulus (e.g. rise in plasma osmolality, drop in blood volume or arterial pressure) that can be shown with in-vivo recordings to trigger firing in SON and mPVN neurons, there is an increase in vasopressin heteronuclear RNA (hnRNA) and messenger RNA (mRNA), and powerful activation of several immediate early genes (e.g. cFos, cJun). While the linkage between the neurotransmitter receptors that convey the afferent signals and the signal transduction that leads to gene activation remains a topic under active investigation, the outcome is ribosomal synthesis of a large precursor molecule (prepropressophysin) composed of a signal peptide, the nonapeptide vasopressin (arginine⁸- vasopressin or AVP in most mammals; lysine⁸-vasopressin in pig), a putative carrier molecule neurophysin, and a 39 amino acid glycoprotein (copeptin or CCP). Packaged in secretory granules by a process involving various sorting mechanisms (below) and budding off the trans Golgi complex network, the secretory gran-

ules are then targeted to sites of release (dendritic or axonal) while the precursor peptide complex undergoes further post-translational processing.

Although the 'classical' notion has been that the secretory granules containing vasopressin are transported for release into the plasma from axon terminals in neurohypophysis, these same neurons can also package and release vasopressin centrally by exocytosis from their dendrites. The latter feature may indicate a 'retrograde' transmitter function, serving an autocrine / paracrine action to regulate the activity of the peptidergic neuron (see Ludwig and Pittman, 2003). As proposed recently (Langraf and Neumann, 2004), this may in fact be an indication of an additional modes of neuropeptide release that affect communication in multiple brain regions.

Another interesting property of these magnocellular (and other) neurons is their co-synthesis and co-localization of various peptides that are encoded by different genes (Brownstein and Mezey, 1986). Of particular interest is how cells package and differentially distribute these different molecules. Some clues come from observations of magnocellular neurons that co-synthesized vasopressin and galanin (Landry et al., 2003). Using double immunogold and in situ hybridization combined with confocal and electron microscope analyses, three subpopulations of secretory granules can be detected: galanin alone, most abundant in granules in dendrites; vasopressin together with galanin, more numerous in the perikarya; vasopressin alone, or with galanin, in granules in their neurohypophysial axon terminals. Thus, vasopressin and other peptides may be partially co-packaged and undergo preferential targeting toward dendrites or axons in the neurohypophysis, consistent with the notion of different autocrine / paracrine and endocrine functions respectively.

Vasopressin: stress-induced role in the hypothalamic-pituitary-adrenal axis.

As mentioned earlier, the immunocytochemical demonstration of 'neurohypophysial' peptides beyond the HNS indicated that vasopressin had a role in other neuronal systems. Indeed, roles for neurohypophysial peptide receptors in higher cognitive functions had long been predicted by De Weid (reviewed in De Weid, 1983). One area involves the hypothalamic PVN where vasopressin is synthesized in two populations of cells: the 'classical' HNS magnocellular neurons, and subpopulations of parvocellular (pPVN) neurons. Within the pPVN, two populations of the latter neurons are functionally associated with the

response to stress. a) "Preautonomic" pPVN neurons project to brainstem 'autonomic' centers and sympathetic preganglionic neurons located in the spinal intermediolateral cell column. These neurons are deemed to mediate a rapid 'neural' response to an acute stress that stimulates sympathetic outflow and the release of adrenalin and noradrenaline from the adrenal medulla, an essential component of the 'fight-or-flight' response. Some of these neurons have been shown to express vasopressin and to contribute to the vasopressinergic innervation to the intermediolateral cell column. b) *Corticotrophin-synthesizing factor (CRF) pPVN neurons*, under resting conditions synthesize CRF for secretion into the pituitary portal plexus from their axon terminals in the median eminence. Subpopulations of these cells also contain 'dormant' vasopressin-synthesizing genes. Challenges to homeostasis and stress activate a variety of pathways that converge upon these CRF neurons, conveying stimulus specificity, releasing transmitters that both alter cell excitability (increased firing to release more CRF) and gene transcription. A sustained stimulation triggers production of vasopressin hnRNA and mRNA, promoting co-synthesis and co-release of CRF and vasopressin into the pituitary portal plexus. Upon reaching the anterior pituitary, vasopressin potentiates CRF-induced release of adrenocorticotropin hormone (ACTH) by binding to specific vasopressin V_{1b} receptors on corticotrophs, activating the inositol phospholipid cycle and protein kinase C, mobilizing calcium from intracellular stores and triggering additional ACTH release.

Vasopressin receptors, social recognition and pair bonding.

Pair bonding in monogamous species is regarded as an example of social motivation. The term monogamy implies a social organization in which a male and female mate exclusively with each other, although extra-pair copulations are not unusual in monogamous species. Recognizing that only 3-5% of mammals exhibit a monogamous social structure as defined by these criteria, one group of species, voles of the genus *Microtus*, have emerged as a valuable tool to investigate the neurobiology of pair-bond formation (reviewed in Young and Wang, 2004).

Prairie voles manifest the classic features of monogamy with males participating in parental care and where intruders of either sex are rejected. By contrast with closely related non-monogamous voles (e.g. Montane voles), prairie voles have a high density of

oxytocin and vasopressin V_{1a} receptors in the accumbens and prelimbic cortex or the ventral pallidum respectively, patterns that are thought to arise from species differences in the respective promoter sequences of the V_{1a} receptor gene (Lim et al., 2004). Interestingly, dopamine receptor antagonists and, in males, blocking V_{1a} receptors in the ventral pallidum or lateral septum inhibits partner-preference formation, factors that support the hypotheses that pair-bonding involves conditioned learning, and that the peptide receptors may actually modulate the role of dopamine in a reward circuitry that is triggered by mating. It is intriguing that pair-bonding behaviour can be induced in a non-monogamous species by using viral vector-mediated gene transfer to overexpress the V_{1a} receptor, and this behaviour can be prevented by pretreating virus-treated voles with a D2 receptor antagonist (Lim et al., 2004). Studies of the molecular genetics of pair bonding are now focusing on microsatellite sequences in the 5' flanking regulatory region of the *avpr1a* gene which could potentially induce altered receptor expression in a preexisting reward circuitry leading to changes in conditioned partner preferences. Curiously and of potential importance are three highly polymorphic microsatellite sequences in the 5' flanking region of the human V_{1a} receptor gene (*AVPR1A*) where variation may be associated with autism (Wassink et al., 2004).

Vasopressin in the circadian system: some uncharted waters.

Most organisms show circadian rhythms in physiology and behaviour. Environmental clues modify the phase and period of these endogenous rhythms. In mammals, the hypothalamic suprachiasmatic nucleus (SCN) constitutes the main biological clock, entraining behaviours (e.g. sleep-wake cycles) and physiological processes (e.g. morning peaks in glucose and cortisol) to the solar day. Many studies confirm the dependency of circadian rhythmicity on the integrity of the SCN, and have now defined the genes and the intricate molecular machinery that endows SCN neurons with an intrinsic rhythmicity. What remains to be clarified are the mechanisms whereby SCN neurons entrain their target neurons into patterns of activity that correspond to the observed physiological and behavioural rhythms.

To address this issue, we have initiated studies in rat brain slice preparations to analyze neurotransmission from SCN to select targets. We have observed that electrical stimulation in SCN can evoke rapid excitato-

ry (glutamatergic) and inhibitory (GABAergic) neurotransmission in neurons within the hypothalamic paraventricular nucleus, or PVN (Cui et al., 2001). Currently we are extending this analysis to the thalamic paraventricular nucleus, or PVT, one of only two known extrahypothalamic targets for SCN neurons. Interestingly, vasopressin is synthesized in a subpopulation of SCN neurons that project their axons to both PVN and PVT, suggesting the possibility that vasopressin may participate in neural communication from SCN to its CNS target neurons. Indeed, experiments by Kalsbeek and colleagues (see Kalsbeek and Buijs, 2002) have established that endogenous vasopressin release from SCN terminals in the area of the PVN inhibits the release of corticosterone. However, as mentioned above, exogenously applied vasopressin usually depolarizes CNS neurons. This apparent contradiction seems to have a solution based on our *in vitro* observations that exogenous vasopressin increases GABAergic inputs to PVN neurons (Hermes et al., 2000). Thus, the current interpretation is that SCN vasopressinergic neurons do not project directly to CRF neurons, but rather indirectly reduce their activity through exciting intermediate inhibitory GABAergic neurons.

SCN-derived vasopressin is at the origin of a diurnal variation in cerebrospinal vasopressin levels (Reppert et al., 1987). Also, vasopressin secretion into the medial preoptic area has an important role in generating the preovulatory surge in luteinizing hormone (reviewed in Kalsbeek and Buijs, 2002). Current interest is a role for vasopressin in SCN's extrahypothalamic projection to PVT. Consistent with observations in other CNS regions, our initial results indicate that PVT neurons respond to exogenous vasopressin with a prolonged membrane depolarization and inward current, likely mediated via G protein-coupled V_{1a} receptor that suppresses a leak potassium conductance. We speculate that this SCN pathway may allow SCN-derived vasopressin to have a role related to sleep-wake cycles.

Vasopressin: a molecule with many homeostatic roles

Vasopressin, a molecule originally associated with a major homeostatic role as an antidiuretic hormone, does indeed have many additional roles. This brief overview has focused on select topics related to its actions in the brain and spinal cord. The author has chosen to profile only some of its cellular and behaviour-related actions, recognizing that there are additional areas of neurobiology (e.g. fever and

temperature regulation) where vasopressin subserves important homeostatic functions. The intention is to leave the reader with the impression that a study of vasopressin and vasopressin receptors does indeed lead in many directions.

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2005 CSCI YOUNG INVESTIGATORS' FORUM AWARD RECIPIENTS

This year's Young Investigators' Forum (YIF), a feature of the Canadian Society for Clinical Investigation/Royal College of Physicians and Surgeons of Canada Annual Meeting attracted a record number of participants this year. MD/PhD students and Clinician Investigator Program (CIP) trainees from across Canada presented a wide spectrum of world class research in both poster format and oral presentations. The meeting was held in Vancouver, certainly part of the attraction, is now in its 11th year. The YIF has become the national meeting for clinician scientists in training, providing an opportunity to mix with peers and compete for prizes. Financial support from the Canadian Institutes of Health Research for student travel make this possible. 2005 award recipients were:



Stephanie Petkiewicz

Stephanie Petkiewicz third year PhD student in the MD/PhD Program at McGill University in the laboratory of Dr. Morag Park at the Royal Victoria Hospital in Montreal. She is currently investigating the effects of dysregulated Met receptor tyrosine kinase signaling on the mammary epithelium and its ability to induce mammary

tumorigenesis. She hopes to demonstrate that the murine mammary tumors generated by the dysregulated Met receptor signals accurately model human breast cancer that overexpresses the Met receptor. Following completion of the MD/PhD Program, she plans to enter a pathology residency and continue with breast cancer research.



Neil Goldenberg

Neil Goldenberg is currently in his fourth year in the MD/PhD program at the University of Toronto, in the lab of Dr. Mel Silverman. His research involves investigating the role of munc13 as a potential contributor to the microvascular and renal complications of diabetes. The lab has shown that munc13 is both upregulated and activated in the diabetic kidney, and that it causes apoptosis in transfected renal cell lines. Recently, they have identified munc13 as an effector of the small GTPase, Rab34. Neil's research has shown that munc13 and Rab34 colocalize with actin at sites of membrane ruffling in phorbol ester-treated cells. His future work will involve determining the significance of this finding as it pertains to macropinocytosis, secretion, apoptosis, and potentially extracellular matrix homeostasis in the kidney. After graduation he is looking forward to starting his career as a clinician scientist.



Mark Kirchhof

Mark Kirchhof is currently in the fourth year of an MD/PhD program at the University of Western Ontario. His PhD work has been done under the supervision of Dr. J. Madrenas. Mark's research interest is focused on the regulation of signaling molecule compartmentalization and oligomerization using different experimental models (CTLA-4, CD14/TLR-4, phosphodiesterase 4B2). He is currently working on elucidating the molecular basis of signalosome assembly and anchoring upon engagement of cell surface receptors, in particular the antigen receptor on T lymphocytes (TCR). Emerging evidence indicates that signal transduction through surface receptors is compartmentalized in space and time using defined cellular microdomains such as lipid rafts. The signalling cascades initiated in response to T cell activation through the TCR via the lipid raft microdomains requires coordinated recruitment and assembly of signalling complexes or signalosomes with cell surface receptors, in a process likely involving cytoskeletal components. The results generated in this project may provide us with a deeper understanding of how signalling is sustained within the T cell. Such an understanding would identify novel targets to modulate T cell responses that are generated by TCR-dependent signalling with potential clinical applicability to different diseases. Upon

completion of his MD/PhD, Mark anticipates entering a residency program in which he can combine his interests in research and medicine, ultimately leading to a faculty position in academic medicine.



Dr. Sarah Woodrow

Dr. Sarah Woodrow, completed her MD degree at the University of Toronto in 2000 and is currently on sabbatical from her clinical training as a resident in Neurosurgery, registered in the Clinician Investigator Program at the University of Toronto. Dr. Woodrow, a recipient of a 2-year RCPSC Fellowship in Medical Education, is undertaking research in surgical education with Dr. Richard Reznick and Dr. Stan Hamstra. In addition to an interest in performance and evaluation of surgical skills, Dr. Woodrow's main research focus is on the influence of sleep deprivation on surgical performance. Upon completion of her neurosurgical training, Dr. Woodrow plans to pursue a career in academic neurosurgery in Canada.



Liam Brunham

Liam Brunham is entering the fifth year of the MD/PhD program at UBC and is completing his doctoral research under the supervision of Dr. Michael Hayden in the Department of Medical Genetics. His research focuses on understanding how specific genes involved in cholesterol metabolism function in different tissues and cell types with regards to the generation of HDL (the "good cholesterol") and risk for heart disease. Liam plans to pursue a career as a clinician-scientist in the field of cardiovascular genetics and to apply fundamental insights into the molecular pathogenesis of disease to improved treatment and diagnostic approaches for patients.



Brienne McLane

Brienne McLane, a student in the MD Plus Program at the University of Calgary is currently enjoying her first year of clinical training. She recently completed her Master's thesis entitled "Towards Insulin Gene Therapy: Hydrodynamic Delivery as an In vivo Screening Tool for Liver-Specific, Glucose-Responsive Promoters" with joint supervision by Drs. Ji-Won Yoon and David Lau. In the future Brienne would like to remain active in both the clinical and research aspects of Medicine.



Prism Schneider

Prism Schneider is a member of the Leaders in Medicine program at the University of Calgary. She recently defended her PhD thesis in Biomechanics and is now a first year medical student. Her research interests involve human locomotion biomechanics with a focus on clinical orthopaedic device evaluation with the use of electromyography.

The 2005 Core Medical Residents Research Awards were presented to Dr. Samuel Assafar from the University of Alberta, Dr. Kerri Novak, University of Calgary, and Dr. Daniel Heng, University of British Columbia at the Annual Meeting of the Canadian Society for Clinical Investigation. These awards, co-sponsored by the CSCI and the Canadian Association of Professors of Medicine are to recognize outstanding research by core medicine residents and to highlight the importance of research participation as a component of the core medical training experience. Here are the abstracts of their award winning research.

